LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE
MEMOIR No. 12

STUDIES ON THE EXO-ERYTHROCYTIC CYCLE IN THE GENUS *PLASMODIUM*

MEMOIR SERIES OF THE LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

Already published

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2. Researches in Polynesia and Melanesia. Parts V–VII (relating to Human Diseases and Welfare), by P. A. BUXTON. (Published November, 1928.)
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STUDIES ON THE EXO-ERYTHROCYTIC CYCLE IN THE GENUS *PLASMODIUM*

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LONDON
H. K. LEWIS & Co. Ltd.
1957

PRINTED IN GREAT BRITAIN BY ROBERT MACLEHOSE AND CO. LTD

PREFACE

There exists today no adequate account of the exo-erythrocytic stages of the malaria parasites. These studies represent an attempt to set out the knowledge of the subject as it stands today together with some new information which might otherwise never reach the interested reader. This memoir cannot be treated other than as an interim report on a subject still swiftly growing after the thirty years in the wilderness (1903–1933). The discoveries of Raffaele, James, Huff, Kikuth, Mudrow, Shortt and Garnham bore abundant fruit and in an amazingly short time. 1954 is not an inappropriate year in which to look back on this twenty years of growth. The broad picture is apparent and the era of consolidation is now at hand.

The whole of these studies have been pursued in the Department of Parasitology of the London School of Hygiene and Tropical Medicine. I owe a debt of gratitude to the two Directors of this Department in my time which can be expressed simply by stating that these words and this work could not have been but for the constant presence of Professor H. E. Shortt and Professor P. C. C. Garnham at my elbow.

Many others have rendered me invaluable assistance and advice. Miss W. Wall and Mr. W. Cooper guided the stumbling steps of the tyro in the early stages. Dr. C. A. Hoare, Dr. J. Williamson, Dr. H. B. Fell and Dr. C. Wilcocks have all given unstintingly of their time and advice. I am greatly indebted to Mr. C. C. Barnard and his staff for bibliographical assistance, and to Miss B. J. Luckhurst for reading the proofs and helping with the compilation of the index.

Finally, for the typing of the manuscript and for her patient encouragement, I owe a deep debt of gratitude to my wife.

R. S. BRAY

December, 1954

Note

This work was completed in December 1953. The only information appearing in 1954, which has been included in this memoir, is that concerning *P. ovale*.

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LIST OF FIGURES

All drawings of parasites made by using a *camera lucida*

As burning fevers, agues pale and faint,
Life-poisoning pestilence and frenzies wood,*
The marrow-eating sickness, whose attaint
Disorder breeds by heating of the blood ; . . .

SHAKESPEARE, *Venus and Adonis*

* = mad, *O.E.D.*

STUDIES ON THE EXO-ERYTHROCYTIC CYCLE IN THE GENUS *PLASMODIUM*

CHAPTER I

DEFINITIONS, GENERAL CLASSIFICATION, TERMINOLOGY

DEFINITIONS

THE genus *Plasmodium* (Marchiafava and Celli, 1885) includes parasitic protozoa which reproduce sexually and by sporogony in an insect host and asexually by schizogony in two cycles in the vertebrate host, one in red blood cells producing pigment and the other in cells other than red blood cells.

The exo-erythrocytic cycle in this genus refers to that asexual schizogony which takes place in cells of the vertebrate host other than red blood cells. These cells are usually fixed tissue cells of one type or another, though there are several notable exceptions to this. In this cycle the parasite does not produce pigment. The term" exo-erythrocytic ", first coined by James and Tate (1937b), has gained general acceptance in reference to this cycle but it is not an ideal term as it defines what the cycle is not rather than what it is.

TERMINOLOGY

The terminology here elaborated and discussed refers only to the exo-erythrocytic cycle. Various terms are in use in the descriptions of this cycle and they will be listed roughly along national lines.

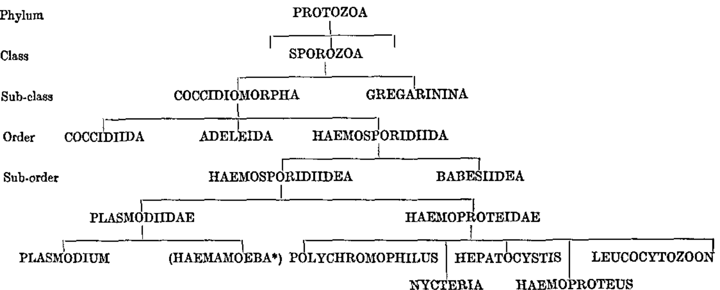
The English terminology derives from James and Tate (1937a) and Shortt and Garnham (1948a).

Exo-erythrocytic or e-e. (Henceforth this abbreviation " e-e " will be used in this work). This term was used by James and Tate to denote schizogony or stages of it in cells other than erythrocytes or reticulocytes. It was later modified by Shortt and Garnham to mean that schizogony in cells other than erythrocytes or reticulocytes occurring after parasitaemia was initiated or was theoretically possible. For the schizogony occurring before parasitaemia they used the term *pre-erythrocytic* or pre-e. (Henceforth this abbreviation " pre-e " will be used in this work.) Terms such as tissue phase or fixed tissue stages are also in use.

The Italian Terminology. Raffaele (1938a, 1938b) referred to the e-e schizogony as the primary monogonic cycle after Grassi. Corradetti (1941b, 1952), Corradetti and Gramiccia (1941b) used the terms :

(a) endo-haemoblastic for schizogony in red cell precursors ;
(b) endo-histiocytic for schizogony in reticulo- and vascular endothelial cells
(c) hepatic endo-epithelial for schizogony in liver parenchyma cells.

The taxonomic position of the genus with its closely allied genera is here accepted as follows :



This formulation combines features from Wenyon, Calkins, Hall and Garnham, and embodies the author's personal views on the phylogenetics of the genus *Plasmodium* (see pp. 153–6).

* The postulation of two genera in the family Plasmodiidae will be made in the last chapter and in the meantime the genus *Plasmodium* will be given its usual present-day scope.

'HAPTER 2

RODUCTION

NOTES ON THE PLASMODIA

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the epidemiology or clinical effects of the

after exo-erythrocytic schizogony are called metacryptozoites. Some of the metacryptozoites, especially after three or four generations appear to penetrate erythrocytes. . . ."

It has been felt that such a definition of metacryptozoites is too indefinite as to the actual duration of metacryptozoite schizogony. The actual appearance of parasitaemia may be after the first metacryptozoite generation or may be delayed months involving scores of generations of the e-e cycle. Therefore the definition has been adopted involving the use of the term " theoretically possible parasitaemia " which has the effect of reducing in fact the number of generations of metacryptozoite schizogony to one at the most in those species of plasmodia adequately studied. The admitted drawback of the definition is that it may be self-eliminatory in that parasitaemia may be theoretically possible after cryptozoite schizogony in all species.

CHAPTER 2

INTRODUCTION

INTRODUCTORY NOTES ON THE PLASMODIA

The plasmodia are exclusively obligate parasites and cause the disease of vertebrates loosely known as malaria. The persistent geographical distribution of the genus in the vertebrate host is apparently confined only by the presence of a suitable insect or definitive host though temperature must play some part. In the vertebrate or intermediate host the biological distribution is widespread, appearing as it does in penguins and toucans, in lizards and in man. Fish are the only class among the vertebrates in which plasmodia have not been described.

Host specificities divide the various species of plasmodia into groups. On the one hand definitive host specificities divide the plasmodia into two groups. The one where the parasites complete their sexual cycle exclusively in anopheline mosquitoes, the other where the sexual cycle may take place in anopheline or culicine mosquitoes. The intermediate host specificities also divide the true plasmodia into two groups. The one group contains those parasitizing vertebrates having nucleated mature red blood cells—the avian and saurian plasmodia. The other group contains those parasitizing vertebrates having non-nucleated mature red blood cells—the human, simian, rodent and chiropteran plasmodia.

These groupings by host specificity coincide, so that we have two main groups.

1. The mammalian plasmodia which are cyclically transmitted exclusively by anopheline mosquitoes.
2. The avian and saurian plasmodia which may be cyclically transmitted by either anopheline or culicine mosquitoes. (This is not proven as yet in the case of saurian plasmodia but may be presumed.)

The phylogenetics of the genus are somewhat obscure. Huff (1938) argues that it may once have been confined to the present insect hosts. There is more convincing evidence, on the other hand, that it represents the furthest remove in a gradual drift away from the coccidia to which it is undoubtedly related.

The disease which these parasites cause has gone under many names, but malaria is now the accepted term. In man it is a disease of the first magnitude, but it is not intended to discuss either the epidemiology or clinical effects of the disease here.

The disease in animals varies widely in intensity in unnatural hosts, but of all natural hosts man is the only one to be constantly and seriously disturbed by the presence of the parasites. There have been isolated reports of heavy infections in some wild birds, and death attributed to the plasmodial invasion, but these are

exceptions (e.g. in blackbirds, Jacobs and Shortt, 1951). On the other hand, there may well be a high death rate among nestlings with plasmodia as a contributory cause. In unnatural hosts, however, some plasmodia can achieve an astonishing virulence.

Domestic animals are remarkably free from plasmodia. The only known outbreak of any importance being that of *Plasmodium durae* in turkeys (Purchase, 1942) though *P. juxtanucleare* can occur in small foci in chickens (Paraense, 1947). Thus the economic importance of the parasites is confined to their effect upon man, in which field they amply compensate for their unimportance elsewhere.

The normal life cycle of a *Plasmodium*, as far as it is known in nature, is briefly and broadly as follows :

A mosquito having in its salivary glands the sporozoites of the *Plasmodium* bites a vertebrate, and the sporozoites are injected intradermally with the mosquito's saliva. The sporozoites are taken up into the general blood circulation and are demonstrable there for 30–50 minutes. They then find their way into fixed tissue cells or cells of the haemopoietic system and reproduce there asexually without the production of pigment. The type of cell parasitized depends on the species of *Plasmodium* involved. This is a schizogonic cycle producing merozoites and takes place once or twice prior to the invasion of erythrocytes. This e-e cycle continues to reproduce itself independently of the erythrocytic cycle as merozoites continue to reinvade the same type of non-erythrocytic cells.

Many of the merozoites from the first or second pre-e generations invade erythrocytes or reticulocytes. In these cells the parasites reproduce asexually by schizogony, producing merozoites which reinvade red blood cells and in the species of group 2 (above) also invade non-erythrocytic cells, thus reinforcing the e-e cycle. This cycle in red blood cells produces pigment.

Some of the plasmodia in the red blood cells differentiate into male and female gametocytes. These sexual forms can fertilize each other and develop fully only in the gut of a mosquito. When a suitable species of mosquito bites the vertebrate having gametocytes in the blood they are taken up into the mid-gut of the insect. Here the male gametocyte produces the gametes which penetrate and fertilize the female gametes forming zygotes. The zygote elongates to form an oökinete which makes its way to the outside of the mosquito's gut wall to come to rest between the gut wall and the membrane surrounding the gut. Here the oökinete reproduces by sporogony to become an oöcyst which eventually contains some thousands of spores or sporozoites. These then break out and the majority come to rest in the salivary glands of the insect. The insect is now infective.

This, as can be seen, is no simple life cycle and only the intense rate of reproduction can explain the remarkably successful survival of the genus.

CHAPTER 3

HISTORICAL

(a) THE GENUS AND THE DISEASE

THE disease caused by the genus in man has been known from time immemorial. It is referred to in the Orphic poems (c. 1,000 B.C.) and in the philosophizings of an early Chinese emperor. It was first described and defined as a disease entity by Hippocrates (c. 400 B.C.). The fevers were described in some detail by the Roman physicians Celsus and Galen.

In the middle ages the quotidian, tertian and quartan agues were well-recognized fevers and were often referred to in contemporary chronicles. Many great men are recorded as having suffered from the disease, especially in the lands around the Mediterranean.

The disease of itself is one which has truly shaped the courses of history, playing as it did an enormous part in any military or social endeavour in the Middle and Far East, in Africa and in the Balkans. The victorious Alexander was turned back from virtual mastery of the land from Hellas to India by the disease. Such a conquest, if consolidated, would have resulted perhaps in an entirely different culture in the Mediterranean basin.

Shakespeare provided us with a less gloomy view of the disease by his use of the fevers as a parallel for that other common disease of man—love: "... for he seems to have the quotidian of love upon him." *As You Like It*, Act III, Scene 2.

In the Americas one wonders whether malaria played its part in "selecting" the Spanish *conquistadores* as the conquerors of the middle and southern portions of those continents. It is known, for instance, that a quartan ague terminated the clerkly career of Hernán Cortéz and sent him in search of more lucrative adventures. At all events, the Spanish conquest of Peru brought about the first relief for the civilized world from the chills and fevers of malaria. In the forests of Peru grew the cinchona tree, whose bark was an efficient febrifuge. About 1630 the bark is said to have cured the wife of the Governor of Peru, one Count of Chinchon, of an intermittent fever. This pleasant tale is probably apocryphal. About 1640 it is believed that a Jesuit father, truly *ad majorem Dei gloriam*, brought the bark to Europe and thus quinine came into general use in the treatment of malaria. In 1820 Pelletier and Caventou isolated the alkaloid in the pure state from the bark and the process was quickly commercialized and equally quickly abused.

In the latter part of the nineteenth century the classic researches of Pasteur and Koch on the aetiology of disease stimulated further studies in malaria with the intention of finding the aetiological agent. This was encompassed by Laveran in

1880, who described amoeba-like bodies in the red cells of patients suffering from malaria. He noted particularly the pigment and the gamete production of the male gametocytes.

Laveran's announcement was not immediately accepted by scientists then obsessed with bacteria identifiable by the carefully laid-down rules of Koch. However, Danilewsky observed similar parasites in the red blood cells of birds in 1885. In the late 1880s and early 1890s Laveran's discovery was confirmed and brilliantly extended by the work of the great Italian school of malariologists including Marchiafava, Bignami, Golgi, Celli and Bastianelli. Where Laveran had been somewhat vague and indecisive, these workers were precise and dogmatic and, all honour to them, remarkably correct. In only one major detail—the fate of the gametocytes—were they in any manner wrong.

The advent of the Romanowsky stains in 1890 greatly assisted the study of the parasites and, by the late 1890s, all three major species infecting man had been described by Golgi and others. Another plasmodium, *P. relictum*, of birds, had also been described by Grassi and Feletti.

In 1897–1898 an important observation was made by MacCallum, working with *P. falciparum*, when he recognized the true significance of the crescents and the "exflagellation" of the male gametocyte. He followed and described the full fertilization process. In 1898 Ross worked out the full sporogonous cycle of *P. relictum* in culicine mosquitoes and gave the world the surprising observation that mosquitoes carried malaria. This observation was confirmed in human malaria by Bignami, Bastianelli and Grassi also in 1898. They noted that it was the *Anopheles* which transmitted the human disease, which was confirmed by Grassi's work at Albanella and Manson's experiments in London and the Roman Campagna.

In the early 1920s malaria therapy of general paralysis of the insane was introduced and the study of the human plasmodia under controlled laboratory conditions was greatly facilitated thereby.

In the realm of antimalarials, 1926 saw the discovery of plasmoquine (pamaquine) and, in 1932, atebrin (mepacrine) was introduced. The discovery of these drugs was facilitated by the use of avian plasmodia, a number of which had been discovered by this time. Techniques for their use in drug assays had been worked out by Roehl, Schulemann and Kikuth in Germany.

In 1933 Sinton and Mulligan in India attempted to rationalize the muddle into which the contemporary knowledge of the simian malaria parasites had fallen.

In the next year Raffaele discovered the existence of the e-e cycle of avian malaria parasites and in 1937 James and Tate established the cycle as a definite and essential part of the life history of the plasmodia. In 1938 Kikuth and Mudrow gave the first description of the pre-e forms of avian plasmodia and, in 1943, Reichenow and Mudrow gave the first full description of the pre-e generations of avian plasmodia.

The e-e cycle in mammalian malaria parasites was destined to remain cryptic until 1948, though Fairley's work in 1944 put the existence of such a cycle beyond doubt.

During the war years proguanil and chloroquine were introduced as antimalarials.

In 1948 Shortt and Garnham uncovered the pre-e cycle of simian and human plasmodia, thus completing the broad knowledge of the biology of malaria parasites.

(b) THE EARLY HISTORY (1890–1934) OF THE EXO-ERYTHROCYTIC CYCLE

Any glance back at the e-e cycle of plasmodia must be cast along one of two lines. One of these, chiefly involving the avian plasmodia, concerns the direct microscopic observation of e-e forms. The other, chiefly concerning the human plasmodia, looks to the various theories advanced to explain the origin of the malarial relapse and the resistance of certain species to radical cure by quinine therapy.

The question of who first saw an e-e form of *Plasmodium* must needs remain shrouded in time. Certainly Danilewsky (1890) described intracellular parasites without pigment in erythroblasts, normoblasts, leucocytes and lymphocytes in the bone marrow of birds. It has been assumed that he was describing *Leucocytozoon* mixed perhaps with *Plasmodium*, though Danilewsky said they were malaria parasites. It should be remembered, however, that he used the word malaria in the loosest possible sense to include most of the Haemosporidiidea. These parasites may have been *Leucocytozoon* or even *Atoxoplasma*, but the possibility that they were e-e forms of *P. elongatum* developing in the bone marrow cannot be dismissed.

MacCallum (1898) provided the first description of what can be taken to be a genuine e-e form of *Plasmodium*. In birds infected with *P. relictum* he described cells in the liver containing 12–30 small oval bodies of the size of avian red blood corpuscle nuclei and staining deeply with haematoxylin. These bodies were pointed and frequently they pointed to the centre of the cluster. He described them in the spleen also but not in the lung or brain. He noted that though they appeared similar to segmenting forms of *Plasmodium* in the blood they did not produce pigment. They occurred only in birds infected with *P. relictum* and not in birds infected with *Haemoproteus*. MacCallum thought them to be protozoan parasites but desired further evidence before associating them with *Plasmodium*. From this description it can be plainly seen that he was dealing with e-e forms of *P. relictum*.

The next relevant event in microscopic examination of *Plasmodium* was an incorrect observation but none the less one of the greatest importance. Schaudinn (1903), after sitting at his microscope for hours to observe this phenomenon,

described in the case of *P. vivax* the direct entry of the sporozoite into an erythrocyte.

Grassi (1900) had written: "Certamente questi sporozoiti, come dimostra il loro nucleo, non sono trasformabili direttamente in sporozoiti delle generazioni monogoniche ordinarie, (cioè, delle generazioni entro il corpo dell' uomo). Deve avvenire perciò una generazione con caratteri particolari." But this correct theorizing was swept away by the announcement of Schaudinn's observations.

Schaudinn's reputation as a parasitologist was such as to bring to an abrupt end any discussion which could provide the theoretical background to such chance observations of the e-e forms which might be, and in fact were, made. This observation then shut the door on research on the e-e phase for thirty years and thus Grassi's theory was stillborn.

The first actual description of an e-e form of a mammalian plasmodium-like parasite was that of Schingareff (1906). He redescribed the *çi-devant* plasmodium of insectivorous bats, *P. murinum*, first described by Dionisi (1899) as *Polychromophilus murinus*, which generic name is now taken to be correct and was used by Schingareff. Schingareff described rare schizonts in leucocytes in the spleen and liver. Some schizonts were noted to contain no pigment. A few forms were also seen in "liver cells", presumably Kupffer cells. He was inclined to look upon them as phagocytosed blood schizonts. As no schizogony of *P. murinus* occurs in the blood, the forms described must have been e-e forms of *Polychromophilus*.

An undoubted description of e-e forms of *Plasmodium* was made by Anschütz (1909, 1910). He was working with a *Haemoproteus*, which he had named *H. orizivorae*, of Java sparrows. In the first paper he described schizogony of *H. orizivorae* in leucocytes and parthenogenesis of gametocytes of *H. orizivorae* into schizonts within red cells. He was obviously dealing with a mixed infection of *Haemoproteus* and *Plasmodium*. The *Plasmodium* was probably what Brumpt (1935) described as *P. paddae*, which, however, is believed to be *P. relictum*. The schizogony seen in leucocytes may have been e-e schizogony, but equally may have been phagocytosis of erythrocytic schizonts. In his second paper Anschütz described schizogony of *H. orizivorae* in brain capillaries and again in leucocytes. In this case he mentioned that *P. relictum* was also present. The accompanying illustrations of the schizogony show perfect reproductions of e-e forms of the *P. gallinaceum* type in the vascular endothelium of brain capillaries. The *Plasmodium* involved is either *P. relictum* or *P. cathemerium*, probably the latter, if in fact the latter is a true species. Huff (1947) believed the parasite to be *P. cathemerium*.

A doubtful record of e-e schizogony is that of Fantham (1910a) who described schizogony of *Leucocytozoon* in the same host cells that the gametocytes were parasitizing. In a later paper (Fantham, 1910b) he described vermicular merozoites in schizonts 14μ wide in cells of doubtful nature in the spleen. Since this is not true schizogony of *Leucocytozoon* it may have been e-e schizogony of *P. elongatum* in red cell precursors.

Another doubtful description of e-e schizogony is that of de Beaurepaire Aragão (1911) who described six species of *Haemogregarina* in various birds. In two of the birds he described schizogony of the haemogregarines in the lung and in leucocytes. The type of schizogony described in the lung is not the *Haemoproteus* type and it seems that e-e schizogony of *P. elongatum* or *P. cathemerium* may have been involved. The possibility that they were *Hepatozoon* should not be overlooked, especially as the type of division was such as to cause Hoare (1924) to remove the two species to the genus *Toxoplasma*.

For yet another dubious case we are indebted to Coles (1914) who described young schizonts of 15–25μ in lung, axillary vessels and in heart blood. He thought them to be schizogonic forms of *Leucocytozoon*. The bird in question was infected with *Plasmodium*, *Haemoproteus*, *Leucocytozoon*, *Trypanosoma* and a microfilaria. The *Plasmodium* infection was heavy. It would seem then that Coles saw schizogony of *Haemoproteus* (Wenyon, 1926), but the possibility that e-e schizogony of the *Plasmodium* was also seen cannot be ruled out in the light of the heart-blood observations.

Carini and Maciel (1916), describing a number of avian toxoplasmas in Brazil, removed all of Aragão's avian haemogregarines to the genus *Toxoplasma* and added a number of species more. Some at least of their descriptions probably refer to e-e forms of *Plasmodium*, as schizogony is described in endothelial cells in the liver, intestine and particularly in the lung.

The question of avian toxoplasmas is even now a somewhat vexed one. As will be seen later in this memoir, the differentiation between avian toxoplasmas and e-e forms of avian plasmodia was the subject for some discussion. The creation of the genus *Atoxoplasma* (Garnham, 1950) removed most of the "intraleucocytic inclusions" from the field of doubt, but some at least of the descriptions of avian toxoplasmas must be regarded with the suspicion that e-e forms of *Plasmodium* are involved. Such records then as Laveran (1900), Mello (1915), Franchini (1923), Pessôa and Corrêa (1929), and Raffaele (1932) give hints that plasmodia may have been the parasites described in some cases rather than toxoplasmas or haemogregarines. I have found that this is flinging too wide a net to catch but bare suspicions, and the field has proved too barren to pursue.

A clear description of e-e forms of *Plasmodium* was that of Ben Harel (1923) who described parasites apparently attached to mononuclear cells in the spleen of canaries infected with *P. relictum*. The illustrations showed unpigmented trophozoites in mononuclear cells. The strain of parasites Ben Harel used has since been shown to be a mixed infection of *P. elongatum* and *P. relictum*. Huff and others have subsequently stated that the e-e forms seen were of *P. elongatum*, though I have been unable to find documentary evidence to show that they could not equally well have been of *P. relictum*. It may be, however, that Huff has examined original material.

Another description of e-e forms is to be found in the original description of

P. elongatum by Huff (1930). Huff noted unpigmented trophozoites in erythroblasts of the bone marrow in canaries following sporozoite infection. He attached no particular importance to this phenomenon, which is not surprising, as the trophozoites described were probably too young to produce pigment even in erythrocytes, and avian plasmodia frequently display a preference for immature red cells (Hegner and Eskridge, 1938).

Also in this year, Uegaki (1930) described among other haematozoa of birds schizogony (*sic*) of *Toxoplasma* in various cells of the lung and liver and chiefly in mononuclear leucocytes. The illustrations show what can reasonably be said to be e-e forms of a *Plasmodium*. Somewhat surprisingly, Uegaki, among 51 infected birds, described 34 as being infected with *Toxoplasma* sp. and only 2 with *Plasmodium* (*relictum*). Though no doubt some were *Atoxoplasma* one would be left suspicious of so many toxoplasmas even without the illustration showing e-e forms.

In 1932 there occurred another unequivocal description of e-e forms of what was then considered to be a *Plasmodium*. Levaditi and Schoen (1932) described the large merocysts in the liver of African monkeys which are the schizogony stages of *Hepatocystis kochi*. Though these authors saw the gametocytes of *H. kochi* in the blood, they did not connect their appearance with the merocysts in the liver. For this reason they created a new genus and species to include the merocystic forms—*Hepatocystes simiae*.

In the following year, Schwetz (1933), while studying *H. kochi* in African monkeys, described extracellular blue bodies, strongly reminiscent of Koch's blue bodies in piroplasmosis, in the bone marrow of infected monkeys. These bodies were round and, when stained with Giemsa, showed as blue studded with red blocks of chromatin. Schwetz was uncertain of the origin of these forms but mentioned that they seemed to represent the hitherto unknown schizogony stages of *P. kochi* (*i.e. H. kochi*). In fact they were broken-off pieces from the merocysts in the liver.

The time has now come to consider the other line of reasoning which led to the study of the e-e forms of *Plasmodium*.

In the late 1920s and early 1930s, malariologists became increasingly dissatisfied with the state of the knowledge of the biology of the plasmodia and with Schaudinn's description of the direct entry of the sporozoite into the erythrocyte. A number of factors contributed to this dissatisfaction and it is now proposed to study the cause and the historical evolution of this state of mind.

Danilewsky (1890) was the first to propose the theory of breakdown of macrophagic acitivity of certain cells to allow haematozoa to develop in non-erythrocytic cells. He postulated the entry by the parasite of a macrophage at a stage of the macrophage's development when its phagocytic powers were still in the process of formation. The entry of the parasite then caused a destruction of the processes of phagocytic development and powers before the macrophage could grow suffi-

ciently to acquire such powers. The parasite was thereby able to grow at the expense of the macrophage and appear in seemingly fully developed macrophages while suffering none of the usual effects of such a habitat.

Bignami (1891) proposed some kind of arrested or even active development of the malaria parasite in white blood cells from which the parasites could later break out to cause the malarial relapses and recrudescences. He also postulated that such a site conferred upon the parasite a resistance to quinine then noted in relapsing benign tertian and in some cases of malignant tertian malarias. This view was also advanced in more detail by Golgi (1893) and again by Marchiafava and Bignami (1894).

A new factor was to enter into this field in which the Italians were attempting to explain biologically the relapse and quinine resistance. In the paper already mentioned Schaudinn (1903) had reported another phenomenom—parthenogenesis. He described, in some detail but with poor diagrams, the evolution by parthenogenesis of a schizont from an unfertilized macrogametocyte of *P. vivax* 48 hours prior to a clinical relapse. He postulated that here was the cause of relapses. The female gametocyte was resistant to quinine and was able to persist in the inner organs more or less indefinitely. At a favourable time it underwent a process of parthenogenesis, thus causing a regeneration of the asexual schizogonic cycle with its consequent clinical relapse. This theory was to so engage malariologists that Schaudinn's other observation, that of the direct entry of the sporozoite into an erythrocyte, was allowed to pass unchallenged for nearly 30 years. The parthenogenesis theory involved the world of parasitology in an extensive controversy for some years to come.

There were now two theories in existence to explain the malarial relapse and quinine resistance. The Italian school had refined their theories into a postulation that some selected resistant asexual forms retired deep into the inner organs to carry on a low-grade multiplication there. These parasites lurked in wait for a suitable moment to restart full-scale schizogony and bring about a relapse.

Few authors were able to confirm Schaudinn's observation of parthenogenesis, though such men as Canalis, Maurer and Grassi supported his theory. Harrison (1909), although he admitted that such cases might be double invasion of a single erythrocyte by a macrogametocyte and a schizont, nevertheless reported a case of parthenogenesis in benign tertian malaria. Mayer (1906) reported parthenogenesis in a *P. cynomolgi* infection. Anschütz (1909) also reported parthenogenesis of *Haemoproteus* but this was fairly obviously a case of *Haemoproteus* and *Plasmodium* in the same cell. Neeb (1910) reported two cases of parthenogenesis.

To these two theories on the genesis of relapses was added yet another. Mannaberg (1894) had described conjugation between immature forms of *Plasmodium* occupying the same erythrocyte. Craig (1910) redescribed this phenomenon and added that the parasites resulting from this rejuvenation were quinine-resistant and phagocyte-resistant forms which could lurk indefinitely

in the viscera until conditions were favourable for their further development and subsequent breakout.

A number of other theories had also been evolved but were not supported to any appreciable degree. Such theories were: encystment of the parasite (Marchiafava and Bignami), resistant sexual bodies (Thayer) and arrested latent schizonts (James).

The theory of low-grade schizogony of selected resistant forms in the inner organs claimed powerful support. Ross and Thompson (1910) gave their support and Bignami (1913) restated the case with skill and conviction using the drug-fast trypanosome strains then emerging as analogies. He further pointed out the notable lack of microscopic corroboration of parthenogenesis. James (1912), in an excellent summary, supported Bignami and discounted both the parthenogenetic and conjugation theories, though he allowed conjugation some contributory place in the scheme of things. Henson (1912) arrived at similar conclusions. Gradually Bignami's theory acquired the bulk of support among malariologists. Ziemann (1913) found no parthenogeneis in innumerable Bass and Johns cultures and accepted Bignami's postulates. Thompson (1917) delivered the *coup de grâce* to the theory of parthenogenesis. He remarked that parthenogenesis was unknown among the Protozoa and in any event should apply to the exogenous, not the endogenous, cycle. More significantly he noted that Schaudinn saw the one or two parthenogenetic forms only 48 hours before a clinical relapse and that such a time would be insufficient for the multiplication of the evolved asexual forms to a number necessary to precipitate a malarial paroxysm. He concluded that such forms were a double-invasion phenomenom.

Acton and Knowles (1914) discarded the conjugation theory after studies on *Haemoproteus*. At the same time they preferred the lung to the spleen as the site of low-level multiplication by analogy with their view of *Haemoproteus* schizogony and the pulmonary complications sometimes attendant upon malaria.

Thus Bignami's theories were left victorious, in command of this field, and indeed they are still held as absolute by some Italian malariologists (Corradetti and Verolini, 1950; Ascoli and Alessandro, 1950, 1951).

In 1924, Yorke and MacFie (1924) stated the theory with some modifications, but added an important rider. They stated that after repeated attempts for years they had been unable to repeat Schaudinn's observation of the direct entry of the sporozoite into an erythrocyte. Also in this paper another observation of prime importance was recorded. The authors noted that while quinine failed completely to cure and prevent subsequent relapses in sporozoite-induced infections with *P. vivax*, it usually completely cured blood-induced infections with this parasite and no relapses ensued. They concluded that quinine failed to affect sporozoites but it was lethal to blood forms.

This selective action of quinine had been noted in *P. relictum* infections by Ed. and Et. Sergent (1921, 1922) but its confirmation in the case of human relaps-

ing malaria brought it into prominence. In any case, the action of quinine in avian malaria is not as simple as was set out by the Sergent brothers.

These then were the first two observations which were to throw doubt on Schaudinn's direct entry observation. Yorke (1924), also observed that the relapse rate in sporozoite-induced attacks of *P. vivax* (57%) was far higher than that of blood-induced infections (2%).

It is at this point that the two courses of malarial history in relation to the e-e cycle begin to converge. The theories concerning relapses began to take account of the possibilities of a third cycle.

Yorke's observations, demonstrated once more by James (1931a), led James to mention the possibility of a third cycle in malaria. He suggested that the sporozoite might invade connective tissue cells and develop there. He drew his ideas from the postulate that sporozoites were essentially tissue parasites occurring as they did in the oöcyst and the cells of the mosquito's salivary glands. James himself, however, was sceptical of his own theory and wrote that Schaudinn's clear and detailed description allowed no doubts in this matter and that a better theory in the face of the known facts was that the invasive sporozoite penetrated erythrocytes, whereas the merozoites merely clung to the outside of the erythrocyte. This faculty rendered the sporozoite immune to quinine. To explain the relapse James postulated, as a possible alternative theory, the entry of the sporozoite into a tissue cell and its subsequent dormancy there until the host cell broke down months later.

Yorke (1931) challenged James's statement that Schaudinn's description admitted no question. His repeated failures to reproduce Schaudinn's observations led him finally to reject the theory of direct invasion of the erythrocyte in any of its postulated forms. Thus the words which two hundred years ago Voltaire used to castigate Fontenelle became the theme of malariologists' thoughts upon the observation of Schaudinn: "*But he was deceived by appearances, an accident to which we are only too liable, whether we make use of microscopes or not.*" (*Micromegas*, Voltaire, 1752.) Later in 1931 James (1931b) appears to have accepted Yorke's view in this matter and became the first advocate of the theory of a third site of development. James thought that sporozoites probably developed in reticulo-endothelial (R-E) cells.

Assendelft (1931) also postulated a third site of development. He believed it possible to demonstrate a local multiplication of the sporozoites at the site of inoculation by the mosquito.

The discovery of pamaquin, a drug specific against gametocytes and having some effect upon relapses when given during the latent period, brought to light more interesting facts. Sinton and Bird (1928) noted that while the drug displayed no action upon the asexual blood stages it definitely lowered the relapse rate of sporozoite induced *P. vivax* infections. Also they demonstrated that while the drug cleared the blood of crescents of *P. falciparum* it had no effect upon the

relapse (recrudescence?) rate. Both the theory of low-grade asexual multiplication and the parthenogenesis theory suffered in these discoveries. James *et al.* (1931) showed that pamaquin had a definite prophylactic action in *P. vivax* infections but did not attack the asexual blood forms.

As to the difference in the action of quinine upon sporozoite-induced and blood-induced infections, the results of Yorke and of James were confirmed by Swellengrebel and de Buck (1931) for *P. vivax*, by Russell and Nono (1932) and Kikuth and Giavannola (1933) for *P. cathemerium* and by Tate and Vincent (1933) for *P. relictum*.

All these facts, collected and collated by the Malaria Commission of the League of Nations, led it to voice in its 1933 report the growing conviction that a third site of development of malaria parasites had yet to be uncovered. The report suggested that some sporozoites absorbed into the blood stream found their way into cells of the R-E system and developed there in a manner similar to *Haemoproteus*. The merozoites resulting from this schizogony then entered erythrocytes. It was further suggested that a few sporozoites entered instead connective tissue cells and remained quiescent there until the host cell broke down. The sporozoite then issued forth to continue its normal development and to cause the malarial relapse. It is not difficult to detect the hand of Colonel James in this postulation and in fact it became known as the James hypothesis. Thus the fate of the sporozoite became the question of the year for malariologists in 1933.

Missiroli (1933) was the first to produce an alternative theory based to any extent on microscopic evidence. Recalling Grassi's theory of a third cycle based upon the chromatin composition of the sporozoite and the erythrocytic trophozoite, Missiroli studied the sporozoite nucleus. He noted that it often consisted of a number of chromatin pieces, frequently quite pronounced in size and character, and he concluded that this was due not to fragmentation or nucleoli but to a real nuclear division. Working with *P. relictum* sporozoites inoculated subcutaneously into canaries, he observed that within 2 minutes the nucleus had undergone rapid division into 4 or 8 chromatin pieces. This form he dubbed the sporocyst. He found that this organism then entirely disappeared within 10 minutes. He held that, like certain bacteria, the route of disappearance was the lymph system. In 1934 (Missiroli, 1934) he enlarged upon this theory. He reported numerous failures to repeat Schaudinn's observations. He postulated that the lymphatics were not only the route by which the sporocyst disappeared from the haemorrhage but also that its subsequent development was situated there. He was, however, unable to demonstrate such a development.

In support of his theory Missiroli mentioned two interesting results of researches in his laboratories :

1. That antibodies produced by erythrocytic forms did not affect sporozoites.
2. That the chromatin of the sporozoites was Feulgen positive, whereas that of the erythrocytic forms was Feulgen negative.

1934 was to see two demonstrations which were of paramount importance in the study of malaria parasites. The first was the discovery by Boyd and Stratman-Thomas (1934), of the non-infectivity of the blood during latency after sporozoite entry in *P. vivax* infections. This fact was later to be confirmed many times over for various plasmodia. For *P. vivax* by Sanctis Monaldi (1935), Sanctis Monaldi and Raffaele (1953), and Fairley (1947). For *P. falciparum* by Sanctis Monaldi and Raffaele (1953), Ciuca *et al.* (1937), Boyd and Matthews (1939), and Fairley (1947). For *P. cynomolgi* by Mulligan (1947), Hawking *et al.* (1948) and Mulligan *et al.* (1949). For *P. gallinaceum* by Henry (1939) and Coulston *et al.* (1945). For *P. relictum* by Raffaele (1936b, 1936c) and Missiroli (1937). For *P. cathemerium* by Kikuth (1935), Warren and Coggeshall (1937), and Kikuth and Mudrow (1938). For *P. berghei* by Vincke and Lips (1950) and Vincke and Peeters (1953).

The second important discovery which will be dealt with in the next chapter was the description of e-e forms of *P. elongatum* by Raffaele (1934a), the importance of which was recognized by him.

Thus malariologists were faced in 1934 with this array of circumstantial and direct evidence in favour of a third and non-erythrocytic cycle in malaria.

1. Schizontocides were unable to produce a radical cure in sporozoite induced *vivax* malaria, whereas they cured blood-induced infections.
2. Pamaquin reduced relapse rates in sporozoite-induced *P. vivax* infections while having no effect upon asexual blood forms.
3. The blood was not infective from about 30 minutes after sporozoite inoculation until 8 days after it in *vivax* malaria.
4. Sporozoites prior to inoculation into the vertebrate host had led an intracellular existence.
5. Schaudinn's observation on the direct entry of the sporozoite into an erythrocyte had not been substantiated despite innumerable attempts.
6. The Feulgen reaction of sporozoites and erythrocytic forms differed. (This was shown later to possess no significance.)
7. Specific antibodies to erythrocytic parasites were inactive against sporozoites.
8. An unpigmented malaria parasite situated in a cell defined as endothelial had been described by Raffaele.

To explain some or all of these facts there were in existence the hypothesis of James and the theory of Missiroli.

(c) THE HISTORY OF THE EXO-ERYTHROCYTIC CYCLE OF THE AVIAN PLASMODIA 1934–1943

Raffaele (1934a) mentioned non-pigmented forms of *P. elongatum* in immature cells in the bone marrow of sporozoite-infected canaries. These forms were de-

virulence of the e-e forms and their resistance to drugs by virtue of their site of development. These researches in Europe led the American workers Warren and Coggeshall (1937) to make a critical experiment on the infectivity of various organs during the pre-patent period of the blood infection. They found that the blood of canaries was not infective from $\frac{1}{2}$ to 72 hours after inoculation with sporozoites of *P. cathemerium*. During this pre-patent period after intramuscular inoculation of sporozoites the site of inoculation was consistently infective when emulsified and inoculated into clean birds. The spleen and liver became infective after 48 hours. After intravenous inoculation of sporozoites these authors found the liver and spleen to be continually infective except for one break at 24 hours. The bone marrow became infective after 48 hours. Histological searches of the tissues involved were negative.

Sicault and Messerlin (1937), summing up this energetic year, expressed their belief that a third cycle exists in malaria and, further, that the chemotherapeutic response of this cycle closely resembles that of the sporogony cycle.

The year 1938 dawned upon a number of conflicting theories and opinions concerning the newly-discovered e-e or X forms. Raffaele had a number of supporters for his contention that these forms were the direct and unadulterated descendants of the sporozoites. Kikuth had shown that the "intra-leucocytic parasites" (*Atoxoplasma*) could confuse the issue and some workers were to believe that the e-e forms were in fact toxoplasmas. Missiroli and his colleagues in his laboratory rejected the theory of the intracellular cyclic development of the sporozoite and held that the X forms were aberrant, able to grow in unnatural host cells due to a temporary breakdown of phagocytic powers and immunity. This theory was later to be refined and fully developed by Corradetti.

Many intermediate positions were taken up by various workers and 1938 became as noted for polemic as for experimental work in this field, especially in Italy where the chief protagonists worked.

In Italy, then, Corradetti (1938a, 1938b) observed that the blood and tissue cycles ran parallel courses and contended that tissue forms could arise from blood forms. Later in the year he put forward his then tentative theory as to the genesis of the e-e forms of *P. gallinaceum*, *P. relictum* and *P. cathemerium* (Corradetti, 1938c). He postulated that the development represented an equilibrium between host immunity and parasite where some parasites were able to break down the phagocytic powers of the R-E system and thus develop there. He held that this was purely fortuitous and therefore these forms should not be treated as an essential part of the life cycle of *Plasmodium*. From these contentions it is obvious that Corradetti (1938d) believed that e-e forms could and did develop from erythrocytic forms.

In later papers Corradetti (1938e, 1938f) strongly advocated the fusion of the two families Plasmodiidae and Haemoproteidae. His arguments were based on the fact that the discovery of a tissue schizogony in *Plasmodium* removed the

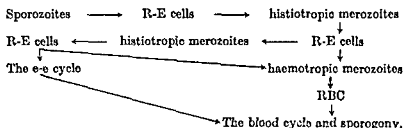
major difference between the families which appeared to contrast with his view of the importance of this schizogony in *Plasmodium*.

In support of Corradetti, Chortis (1938a, 1938b, 1938c) held that e-e development was an accidental phenomenon resulting from loss of phagocytic function due to intense proliferation of the R-E system which he observed. On the other hand, *in chronic infections of P. gallinaceum he observed no relation in intensity* between e-e forms and erythrocytic forms. Decourt and Schneider (1938a, 1938b) also took this view of e-e forms as at that time no forms had been described during the pre-patent period. Missiroli (1938) redescribed his theory of sporozoite development with some refinements. He held that the "sporocyst" (=sporozoite) divided into 8 sporozoites in the lymphatics. These sporozoites then entered red cells *or* R-E cells and developed there. This last admission was forced on Missiroli by the findings of Kikuth and Mudrow to be described later.

All these views met with the opposition of Raffaele and Verney. Raffaele (1938a) held to his views that all e-e forms derived directly from sporozoites and that all the Plasmodiidae would be found to have an e-e cycle. He also introduced the following scheme of development:

The pre-e or primary monogonic cycle arising from sporozoites in R-E cells gives rise to two types of merozoites, the one histiotropic, the other haemotropic. *Succeeding e-e generations continue in the same manner. In support of his contentions* he noted that e-e forms had been seen 72 hours after sporozoite inoculation when blood forms had not been microscopically demonstrable and that, whereas e-e forms were invariably found in sporozoite-induced infections they were not consistently found in trophozoite-induced infections.

Later Raffaele (1938b) produced the following scheme, taking into account the work of Kikuth and Mudrow:



He apportioned the merozoites of the second and successive generations thus:

Species		*haemotropic*	*histiotropic*
P. elongatum	sporozoite-induced	50%	50%
P. gallinaceum	sporozoite-induced	50%	50%
P. gallinaceum	blood-induced	90%	10%
P. cathemerium	sporozoite-induced	50%	50%
P. cathemerium	blood-induced	80%	20%

He claimed that the low figures for e-e forms in blood-induced infections were due to the inoculation of only low numbers of e-e forms in wandering macrophages.

These views were supported by Verney (1938c, 1938d) whose review (1938d) is a useful survey. Verney (1938a, 1938b) disagreed with Corradetti on the classification of the Plasmodiidae. He held the view that the schizogony of *Plasmodium* in erythrocytes was sufficient in itself to preserve the distinction from the Haemoproteidae. At the same time he felt that a new genus was needed to contain those species in which e-e forms had been described.

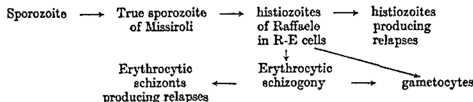
Powerful support for Raffaele was to come from Germany where Kikuth and Mudrow (1938a, 1938b) were to publish the first of many brilliant researches on the pre-e cycle in avian malaria. They were able to repeat the observations of Warren and Coggeshall on the infectivity of organs when the blood was not infective after sporozoite infection. They went on to give the first demonstration of pre-e forms of *P. cathemerium* from 48 hours onwards after sporozoite infection. Though they found the blood to be infective at this time interval this was probably due to cryptozoic merozoites and the forms seen were none the less pre-erythrocytic in all that that implies. These metacryptozoites described by the German workers in 1938 were for the most part mononucleate and intracellular although some extracellular forms were seen and some forms were noted with up to 8 nuclei. Kikuth and Mudrow concluded that the sporozoites invaded R-E cells on entering the body and continued their development there. While agreeing with Raffaele on this point they remained uncommitted on the point of possible genesis of e-e forms from erythrocytic parasites.

It must be stressed that their paper in 1938 stands as the first description of pre-e forms of malaria parasites. This stress is made necessary by the tendency among British workers to ascribe the discovery jointly to Mudrow (1940) and Shortt *et al.* (1940). Even before 1940 Kikuth and Mudrow (1939) were to describe pre-e forms of *P. cathemerium* as early as 16 hours after sporozoite entry, while the observations of Shortt *et al.* were made 5 days after sporozoite invasion.

The central question now became the fate of the sporozoite and the genesis of e-e forms. Rodhain (1938a) described a natural infection of *P. relictum* in penguins at the Antwerp Zoo including an intense e-e development in the brain and elsewhere. He argued that this infection was patently sporozoite-produced and natural, and therefore inclined to the views of Raffaele. In two later papers, however, Rodhain (1938b, 1939) showed that the e-e forms also appeared in penguins following blood passage of the infection and, consequently, withdrew his former assertions.

Two attempts were made to find compromise theories involving part or whole of the positions taken up by Raffaele, Corradetti and Missiroli. Froés (1938) and Sicault and Messerlin (1938) independently produced the following

marriage of ideas which, they felt, fitted the observations of Raffaele, Corradetti and Missiroli, and Kikuth and Mudrow:



This plan is interesting today as it reflects the views on malaria still held by some French workers (Decourt, 1949a).

This scene shall be returned to later, but in 1938 yet another stone was hurled into these already turbulent waters and it as well to dispose of this new site of activity now.

Hegner and Wolfson (1938a) reporting forms in *P. cathemerium* and *P. nucleophilum* infections (the latter proved to be a mixed infection) similar to those seen by James and Tate in *P. gallinaceum*, gave them the name toxoplasma-like bodies, and expressed their belief that they were not forms of *Plasmodium*. They extended their observations later to include *P. relictum* (Hegner and Wolfson, 1938b).

James and Tate revealed that special precautions had been taken against accidental infections and that some of their experiments had been carried out on birds bred by Bishop in such a manner as to be free from protozoa from the time of hatching. Influenced perhaps by this and the weight of other opinion, Hegner and Wolfson (1938c) began to express doubts about the identities of the toxoplasma-like forms and to come around to the belief in plasmodial origin.

In the meantime, this view had brought about some discussion and some misgivings. Raffaele (1938c) rejected the view pointing out that his observations were made on laboratory-bred birds infected by mosquitoes in which no exogenous development of *Toxoplasma* was known. In an excellent review of the recognized intracellular parasitic protozoa of birds Raffaele (1938d) demonstrated that he was well acquainted with *Toxoplasma* and the intra-leucocytic toxoplasms (*Atoxoplasma*) and showed that the e-e forms bore no resemblance to such bodies. Giovannola (1938a, 1938b, 1939) supported Raffaele and differentiated between the e-e forms of *P. gallinaceum* and other intraleucocytic protozoa. Taddia (1938) took the opposite view and pronounced the forms *Toxoplasma*.

Manwell (1939) and Manwell and Goldstein (1939) went into the question of morphology of the two genera in question with great thoroughness and eventually dismissed the notion that the forms seen could be *Toxoplasma*. Manwell pointed out that the bodies seen were round or oval, parasitized all R-E cells and reproduced by schizogony, whereas *Toxoplasma* was crescentic, confined to leucocytes

and monocytes and reproduced by binary fission. Though there is some confusion here (which is not yet cleared up) the views are none the less valid. Wolfson (1940c) eventually expressed her definite conclusion that the bodies were plasmodial in origin.

Ed. Sergent (1940) raised the question again when he described e-e forms in a quail in Algeria which showed neither *Plasmodium* nor *Haemoproteus* in the blood after the most painstaking examination. What was seen, however, were long vermicular uninucleate parasites free in the lung and liver which he took to be toxoplasms. Two varieties of typical e-e forms were described in R-E cells and represented by Sergent to be schizonts with up to 55 nuclei and sexual forms with hundreds of small chromatin dots. Sergent felt that all these parasites represented forms of one species—a species of *Toxoplasma*. In 1941 he redescribed these forms and admitted he was unable to make up his mind about the e-e forms in R-E cells (Sergent, 1941). This case still remains an unsolved problem (Sergent, 1949b). There is considerable flux even now over the question of avian toxoplasms, some authors recognizing three different races or species in birds, but, by 1940, all workers had accepted the e-e forms seen in *Plasmodium* infections of birds as being forms of the relevant *Plasmodium*.

The significance of these forms, however, was still hotly debated. The three main points of contention may be listed thus :

1. The immediate fate of the sporozoite.
2. The genesis of e-e forms, i.e. whether exclusively from descendants of the sporozoite.
3. Taxonomic position of the Plasmodiidae and the Haemoproteidae.

It is intended now to follow these points to such conclusions as are available within the period 1933–1943 before continuing to describe other discoveries made in the period.

1. The immediate fate of the sporozoite

Raffaele contended energetically that the sporozoite, within an hour after inoculation, entered cells of the R-E system and developed there. This of course was the former hypothesis of James and in this he was supported by James, Brumpt, Kikuth and Mudrow, and opposed by Missiroli and Corradetti. Raffaele (1939) added to his theory the postulation of histiotropic or tissue-invading merozoites and haemotropic or erythrocyte-invading merozoites which arose from the tissue phase initiated by the sporozoite. Later Raffaele (1940b, 1941a, 1942) postulated four cycles of development for *Plasmodium*, three of which were obligatory. These three were the cycles of sporogony, pre-e, and e-e schizogony in R-E cells, and erythrocytic schizogony. The fourth, optional, cycle was that of a haemoblastic cycle such as was displayed by *P. elongatum*. It should be remembered that he believed the e-e cycle of *P. elongatum* to be situated mainly in R-E cells.

In opposition to these postulations Missiroli (1939, 1940, 1941, 1941a, 1943) had fully developed his own theory of the fate of the sporozoite. He maintained that the sporozoite, in reality a sporocyst, divided within 12 hours at the site of inoculation to form 4 tear-shaped sporozoites. Further development of the sporocyst took place extracellularly in the lymph system. In 24 hours the sporocyst, containing up to 8 sporozoites, found its way to the spleen and burst, releasing the sporozoites. The sporozoites then invaded erythrocytes to initiate the blood cycle. Some, however, may have invaded tissue cells to initiate " this well known cycle to which belong the parasitic forms which other authors have erroneously described as stages of development of sporozoites ". Endo-erythrocytic and endohistiocytic parasites were reported by Missiroli at 24–48 hours after inoculation of sporozoites of *P. relictum*. The admission that early tissue invasion does occur was wrung from Missiroli by the discoveries of Kikuth and Mudrow, but he refused to accept those forms described prior to 24 hours. Missiroli was supported by Archetti (1941) who tested the action of drugs upon the " sporocystic forms " and partially by Corradetti (1940a, 1940b, 1940c, 1940d, 1940i, 1941a) who questioned the significance of the non-infectivity of blood in the prepatent period, asserting that it was merely a question of the amount of sporozoite inoculum used.

General opinion and experimental evidence were mounting against Missiroli. James (1939) reasserted his original theory but the experimental evidence was to come from the painstaking fundamental research of the German workers Kikuth, Mudrow, Schulemann and Reichenow.

Kikuth and Mudrow (1939a, 1939b) described stages of the pre-erythrocytic generations of *P. cathemerium* from 16 hours to 64 hours after intra-muscular inoculation of sporozoites from up to 100 *Culex*. These forms were found in the majority of cases to lie intracellularly at the site of inoculation and to be undergoing active schizogony in histiocytes of the R-E system. The authors accepted this as final proof of the intracellular development of the sporozoite. Mudrow (1940) reported that the first pigmented erythrocytic parasites to occur were seen 96 hours after sporozoite inoculation. These findings were supported by the opinions of Verney (1939), Filippini (1939), Giovannola (1939), Porter and Huff (1940), Schulemann (1940) and Porter (1940) and by the experimental work of Shortt *et al.* (1940) who showed that the e-e forms of *P. gallinaceum* appeared before blood forms were demonstrable by microscopic examination, and of Schulemann and Spies (1940) who repeated the observations of Kikuth and Mudrow using a special technique to stimulate histiocytic activity at the site of inoculation

Kikuth and Mudrow (1940) repeated their observations with *P. gallinaceum* and extended their observations on the pre-e cycle of *P. cathemerium* back to 9 hours. They, however, had not yet differentiated between the two pre-e generations, but they did postulate that haemotropic merozoites were not produced

until after one or two generations of histiocytic development. Kikuth and Mudrow (1941a) put on record their views upon the whole question of the tissue phase in the first one of their long, exhaustive and excellent reviews which they have published together or separately from time to time ever since. In this review they uncompromisingly stated that the sporozoite from the mosquito's glands developed intracellularly and, later in the year, they detailed their objections to the Missiroli hypothesis. These objections were chiefly contained in their own precise observations from 9 hours after inoculation, but they also pointed out that the so-called division of the sporozoite nucleus does not appear when wet fixation of the bodies is properly carried out.

Mudrow (1942a, 1942b) extended her previous observations to include forms seen from 5 hours to 18 hours after inoculation of sporozoites of *P. cathemerium*, *P. relictum* and *P. gallinaceum*. She found that for up to 5 hours the sporozoite lies free and then invades R-E cells in all three species. She described multinucleate forms at 18 hours at the site of inoculation (breast muscle) and she was now coming to believe that two generations were involved in the time observed (up to 65 hours). In the meantime Schulemann (1942) had reconsidered his previous findings and, on further experimentation, he decided that he could not fully support either the views of Missiroli or Mudrow. His doubt was occasioned by the finding of extracellular quadrinucleate forms 24 hours after inoculation.

Confirmation of the German work came at last from South America where Paraense (1943) found intracellular schizonts of *P. gallinaceum* in the scrapings of the dermis from the site of sporozoite inoculation. These were observed from 18 to 84 hours after inoculation and the first endo-erythrocytic forms were seen at 84 hours. Huff, Coulston and Cantrell (1943) also gave hints of similar confirmatory research (done under conditions of war-time secrecy) by their coining of the word " cryptozoite " to describe first generation e-e forms. Finally, Reichenow and Mudrow (1943) published their classical paper on the development of the sporozoites of *P. relictum*, which placed the question of the fate of the sporozoite beyond doubt and established the existence of the two pre-e generations later to be named the cryptozoite and metacryptozoite schizogonies.

2. The genesis of the e-e forms

The second large question mark of the time was whether the e-e cycle could be initiated by the erythrocytic merozoites or whether it must arise exclusively from the tissue-inhabiting descendants of the sporozoite. The two main protagonists in this debate were Raffaele who supported the latter view and Corradetti who took the former view. Raffaele (1938a, 1939, 1940a, 1940b) took the stand that all e-e forms were derived from the sporozoite and subsequent e-e forms without recourse to blood forms. He stated that only the histiotropic merozoites of the e-e cycle could continue to invade tissue cells and, therefore, the e-e forms appearing after blood-passage of *Plasmodium* were derived from the scanty e-e forms

seen in wandering macrophage cells in the blood. This, he said, would explain the delay in the appearance of an e-e cycle as compared with erythrocytic forms in blood-induced infections. James (Raffaele, 1951) had already warned against this view and later he gave it as his opinion that it was too much to expect that a chance inoculation of an e-e form should occur in 70 per cent. of blood-induced infections and that such a form should be virtually dormant for 10–12 days and then suddenly begin a swift multiplication producing thousands of merozoites in such a short space of time. He concluded that the evidence pointed to the probable genesis of e-e forms from erythrocytic forms which were in a position to supply the numerous merozoites necessary to produce the sudden appearance of large numbers of e-e forms characteristic of the blood-induced infections of *P. gallinaceum* in older chicks. Some support for Raffaele's claims came from Kikuth and Mudrow (1938b, 1939c) but these authors were to express their neutrality in this matter in the following two years (Kikuth and Mudrow, 1940, 1941a).

The main weight of evidence against Raffaele's ideas and in favour of the initiation of the e-e cycle by erythrocytic forms came from workers at the Istituto di Sanità Pubblica in Rome, the foremost being Corradetti. Corradetti (1940a, 1940b, 1940c, 1940d, 1940e, 1940g, 1940j, 1941a, 1941b, 1941c, 1941d, 1941e, 1942a, 1942c, 1943) dealt with the question at some length. Leaving aside for the moment his theory of host-parasite balance, Corradetti postulated the initiation of the e-e cycle from blood forms, basing his assumption upon experimental data collected on blood-induced infections of *P. gallinaceum*. His findings can be listed thus:

(1) The appearance of e-e forms is transitory, lasting from 11 to 25 days after infection and only very rarely more or less.
(2) E-e forms appear in birds infected with a strain repeatedly blood-passages on the fourth day after infection of the donor, a time when e-e forms are not visible. This holds true even in a strain blood-passaged daily.
(3) In scrupulous examination of inoculated blood in the above cases no e-e forms have been seen in wandering macrophages. This applied to a series of 87 passages.
(4) E-e forms are retarded by an equal period of time as erythrocytic forms on treatment with chemotherapeutic agents.
(5) E-e forms do not appear until endo-erythrocytic parasites are being phagocytosed whole instead of merely pigment, i.e. on or about the 12th day after infection.

These represented a formidable array of observations even if (1) and (5) are not strictly true and (4) is completely wrong. One or other of these observations and Corradetti's view as a whole were supported by the work and opinions of Mosna (1940) Ritis (1940), Taddia and Viero (1940), Schulemann (1940) and Porter (1942).

Zain (1941a, 1941c) in an attempt to settle this question, centrifuged infected blood at high speed and found the supernatent fluid contained erythrocytic schizonts broken out of red cells. These proved to be infective and the resultant infection showed e-e as well as erythrocytic forms. This, however, was not a crucial experiment, but such a one was to come from the United States.

The question was put beyond doubt by Manwell (1940) and Coulston and Manwell (1941) who were able, by micromanipulation or by serial dilution methods, to isolate and inoculate a single erythrocytic parasite. The strain thus initiated, after several passages, produced e-e forms. This was crucial and it was thus finally proven that e-e forms could develop from erythrocytic forms. Hewitt (1941) thought that such an invasion of tissue by blood-cycle merozoites was assisted by the clumping of the parasitized red cells around endothelial cells which he had observed *in vitro*.

3. Taxonomic positions of the Plasmodiidae and the Haemoproteidae

The third major question of the time, the problem of the revision and redefinition of the families Plasmodiidae and Haemoproteidae, remains unsettled even today, though much progress has been made. The discovery of an e-e cycle in *Plasmodium* demanded of zoologists a review of the whole classification of the Haemosporidiidea, removing, as it did, the plasmodia from their position of isolation among the Coccidiomorpha when conferring upon them a tissue site of development.

James and Tate (1937a, 1937b) were the first to call attention to the need for taxonomic review and the necessity for a new definition of the genus *Plasmodium* and consequently of the family Plasmodiidae. They pointed out the similarity between the newly discovered e-e forms of *Plasmodium* and the schizogony phase of *Haemoproteus*. Missiroli (1937a) suggested the creation of a new genus *Istiocytozoon* to include the former plasmodia which were shown then or later to display e-e forms. It was at the time to include *P. gallinaceum* and *P. elongatum*, both of which had been definitely shown to have a tissue stage in their development.

Corradetti (1938d, 1938e, 1938f, 1940g, 1941d, 1942a, 1942c, 1943) held that the two families involved should be fused and therefore that *Plasmodium*, *Haemoproteus* and *Leucocytozoon* should be considered as member genera of the family Plasmodiidae. He believed that the e-e cycle in *Plasmodium* is the last evolutionary stage of a general shift away from the Eimeriidea within the Coccidiomorpha. He felt that the erythrocytic schizogony of *Plasmodium*, which he describes as merely a degree of adaptation, though sufficient to differentiate a genus, was not sufficiently significant a phenomenon to differentiate between families now that other characteristics had been found to be similar in this case. This view was shared, at least in its conclusions, by Giovannola (1939) and, with reservations, by Manwell and Goldstein (1939).

The opposite view was taken by Verney (1938a, 1938b) who held to separatist

views. He believed that the existence of an endo-erythrocytic schizogony in the Plasmodiidae was sufficient in itself to retain this as a family separate from the Haemoproteidae. He also rejected Missiroli's suggestion for a new genus. Porter and Huff (1940) and Huff (1942) remained noncommittal while noting that the state of affairs was not satisfactory. Carpano (1942) noted certain points of similarity between the Plasmodiidae and the Piroplasmidae but he made no suggestions for taxonomic changes.

Leaving the three points upon which so much recondite polemic was lavished, it would be well to record the experimental data and new facts about the avian e-e cycle which accrued in these years, apart from those heretofore mentioned.

In the field of chemotherapy, James and Tate (1937, 1938), Kikuth and Mudrow (1939c) and Ritis (1940) showed that quinine, mepacrine and Certuna had no effect upon e-e forms. Mudrow (1940) found that pamaquine showed some activity against these stages. Corradetti and Gramiccia (1943) showed that quinine and mepacrine were ineffective against the non-pigmented stages of *P. elongatum* circulating in the peripheral blood.

About the site of development of the e-e stages of *P. elongatum* some argument had arisen. Raffaele (1936a, 1937a, 1938a, 1938b) held to his view that these stages were to be found in R-E cells, whereas Huff and Bloom (1935) had described them as largely inhabiting cells of the erythrocyte-forming series. Raffaele was supported by Verney (1938a, 1938b, 1938c, 1939) but came under heavy fire from Corradetti (1938c, 1938d, 1938f, 1940d, 1940e, 1941e) and Corradetti and Gramiccia (1941a, 1941c) who went further than Huff and Bloom to assert that all e-e stages of *P. elongatum* were to be found in cells of the haemopoietic system. Porter and Huff (1940) repeated the results of Huff and Bloom in that though the forms were to be found largely in erythrocyte-forming cells, a small proportion none the less appeared in fixed tissue and wandering macrophages; there the matter stands as Raffaele (1940b) allowed that such a site of development did exist.

The knowledge concerning the e-e cycle of *P. gallinaceum* was increased by many discoveries. Jacobi (1939) found that the e-e forms appeared from the 25th to the 30th days after intramuscular inoculation of infected blood. These times were modified to 11–30 days after intravenous inoculation by the work of Ungo-Mugdan (1939), James (1939), Corradetti (1940a, 1940c, 1940d, 1940e, 1940g, 1940j, 1941a, 1941b, 1941c, 1941d, 1942b, 1942c, 1943), Ritis (1940), Mosna (1940), Launoy (1940) and Zain (1941b). Many of these authors also showed that, on sporozoite infection, the e-e forms appeared much earlier.

The infection by emulsions of tissues containing e-e forms was carried out by Villalobos (1940a, 1940b), Mosna (1940), Zain (1941b) and Adler and Tchernomoretz (1941) and was shown to produce a pattern of infection similar to that of sporozoite inoculation. Adler and Tchernomoretz (1941) carried the infection by e-e forms through 5 passages without the appearance of blood forms. Corradetti

(1942b) confirmed these results, using turkey poults as well as chickens. Mosna (1940) and Corradetti (1942b) demonstrated that blood taken at the time of e-e development produced an infection akin to that produced by sporozoites or e-e forms. James (1939) showed that while 95 per cent. of sporozoite-inoculated chicks produced e-e forms, only 54 per cent. of blood-inoculated birds produced e-e forms if able to survive the primary acute parasitaemia.

The actual number of e-e forms developing was found by Schulemann and Knoche (1941) and Knoche (1941) to be greater if R-E proliferation was induced by intravenous inoculation of dyes. A similar result was supposed by Zain and Wolf (1943) to ensue after X-irradiation.

Corradetti (1940c, 1940e, 1940g) claimed to be able to delay the appearance of e-e forms after blood inoculation by temporarily eradicating the blood forms with quinine. Adler and Tchernomoretz (1941), however, found that e-e forms obtained by combined blood inoculation and quinine treatment appeared earlier and in greater numbers than in untreated blood-induced infections. Although Adler and Tchernomoretz undoubtedly used heavy inocula and young birds whereas Corradetti, producing as he was chronic infections, must have been employing older birds and light inocula, it must be said that Corradetti's results are not the general rule and that the use of quinine does not normally delay the advent of e-e forms.

The distribution of e-e forms of *P. gallinaceum* on intravenous blood or sporozoite inoculation was investigated by Brumpt (1937) and James and Tate (1938), and of pre-e forms on intravenous and intramuscular inoculation of sporozoites by Kikuth and Mudrow (1941b) and Schulemann and Spies (1940). Brumpt found e-e stages in the reticulo-endothelium and vascular endothelium of the brain, kidney, lung, myocardium, spleen, bone marrow and liver and rarely in the leucocytes. Kikuth and Mudrow found pre-e forms in liver, spleen and lung on intravenous inoculation of sporozoites and in histiocytes at the site of inoculation after intramuscular injection of sporozoites.

Tissue culture of e-e forms of *P. gallinaceum* was attempted by Gavrilov *et al.* (1938) and Rodhain *et al.* (1940), without complete success.

The e-e forms of *P. relictum* first described by Raffaele (1936b) were seen in nature by Rodhain (1938a) and Taddia and Viero (1940), and in the laboratory by Kikuth (1937a), James and Tate (1938), Hegner and Wolfson (1938b), Rodhain (1938b, 1939), Corradetti (1938d, 1941g), Manwell (1940) and Dobler (1941). The pre-e forms were described by Mudrow (1942a) and Reichenow and Mudrow (1943). The distribution of e-e forms of *P. relictum* was described by Kikuth (1937a). He found them in R-E cells of the brain, liver, spleen, kidney, heart and bone marrow. Manwell (1940) observed that *P. relictum* e-e schizonts are usually vacuolated.

The e-e forms of *P. cathemerium*, first described by Kikuth and Mudrow (1937), were noted also by Corradetti (1938a, 1938b, 1941h), Hegner and Wolfson (1938a, 1938b), Wolfson (1940a), Hewitt (1940b) and Porter (1942). The pre-e

forms of *P. cathemerium* were demonstrated by Kikuth and Mudrow (1938b, 1939a, 1939b, 1939c, 1941a, 1941b), Mudrow (1942a) and Schulemann (1942). The distribution of e-e forms of *P. cathemerium* was studied by Porter (1942) who found them early in the infection in R-E cells of the liver, spleen and bone marrow and later in vascular endothelium throughout the entire vertebrate host body.

E-e forms of *P. circumflexum* were described by Manwell and Goldstein (1939) and were found (in order of distribution frequency) in the lung, brain, spleen, liver, bone marrow, heart muscle and ovaries. The host cells were described as lymphoid-macrophage cells. This work was confirmed by Coulston and Manwell (1941) in their single parasite infections experiments previously described.

The e-e forms of *P. durae* were demonstrated by Purchase (1942) in the lung, spleen and brain of turkeys.

The e-e forms of *P. juxtanucleare* were described by Barretto (1943) in the brain of chickens.

These six plasmodia referred to above all showed a similar type of e-e development.

Some failures in the search for e-e forms were reported. Hegner and Wolfson (1938b) had searched in vain for the e-e forms of *P. oti* (=*P. polare*), *P. rouxi* and *P. vaughani*. Curd (1943) noted that *P. rouxi* seemed to be the only avian plasmodial infection which could be completely sterilized by schizontocidal drugs following blood infection. This gave the impression that in blood-induced infections the e-e cycle of this parasite may not develop. Hegner and Wolfson (1938a, 1938b) had reported positive findings of e-e forms in blood infections with *P. nucleophilum*. These descriptions appear to have been the result of mixed infections of *P. nucleophilum* and *P. relictum* and it is generally accepted that e-e forms of *P. relictum* were in fact described. Goldstein (1939) and Manwell and Voter (1939) reported failure to uncover the e-e forms of *P. nucleophilum*. Terzian (1941) reported similar failure in the case of *P. lophurae*.

Porter and Huff (1940) pointed out that one of the avian plasmodia stood out by itself when the e-e cycle was considered. *P. elongatum*, as had been shown by Huff and Bloom (1935) largely parasitized cells of haemopoietic origin, and the number of merozoites produced by the e-e cycle of this species was considerably less than those produced by the other six species in which the e-e cycle was known. Wolfson (1940b) succeeded in producing e-e forms of *P. elongatum* in duck embryos. Corradetti and Gramiccia (1941a, 1941c) pointed out that while e-e infections of *P. gallinaceum* are often transitory after blood inoculation, the e-e infections of *P. elongatum* not only persisted but represented the main life and growth of the parasite.

During this period, 1934–1943, some general summaries concerning the e-e cycle were published. Such were : Verney (1938), Giovannola (1939), Porter and Huff (1940), Hewitt (1940a), Mudrow (1940), Raffaele (1940b), Sergent (1940),

Schulemann (1940), Kikuth and Mudrow (1941a), Sergent (1941) and Corradetti (1943).

(*d*) THE HISTORY OF THE EXO-ERYTHROCYTIC CYCLE OF THE MAMMALIAN PLASMODIA, 1934–1947

Much of the theoretical discussion concerning e-e forms in malaria reported in the last section embraced, in the minds of the theoreticians involved, both the avian and mammalian malaria parasites. Thus, Missiroli's theory of sporozoite development was held by him to be true for all species of *Plasmodium*, as were the postulations of Raffaele and of Kikuth and Mudrow, based as they were upon the James hypothesis first brought into being to explain anomalies in the knowledge of human malaria. However, as the e-e forms of mammalian plasmodia remained cryptic during the period to be discussed, the theoretical ground covered in consideration of e-e forms was largely directed towards the explanation of relapses, the action of drugs and the inevitable comparisons with the discoveries in the avian parasites. This is not to say that reports of demonstrations of the cryptic forms were not made. Indeed they were, but in no case were they sufficient to allow the building around and upon them of a coherent scheme of e-e schizogony. Thus, though many workers believed, upon theoretical grounds and by analogy, that an e-e cycle existed in mammalian malaria (James, Nicol and Shute, 1936; Ruge, 1936a, 1936b; James, 1937; Raffaele, 1937b, 1939, 1940b, 1941, 1942, 1944a, 1944b, 1946a; Kikuth, 1938, 1939, 1943; Reichenow, 1939; Davey, 1944, 1946b; Taliaferro, 1944, 1947; Fairley, 1946a, 1946b, 1947; Shute, 1946; v. Haller, 1947; Boyd, 1947) no definite microscopical proof of such a cycle was forthcoming in these years.

The theoretical grounds for this belief may be restated.

1. The period of pre-patency.
2. The differential action of schizontocides in sporozoite- and trophozoite-induced *P. vivax* malaria.
3. The relapses after complete inactivity in *P. vivax* and *P. malariae* malaria.
4. The existence of an e-e cycle in avian malaria.

The period of pre-patency had been shown to exist and its limits defined by blood subinoculation following sporozoite infection. (Chap. 3, Section (*b*).)

The differential action of the schizontocides was confirmed for *P. vivax* by Ciuca *et al.* (1937b) and by Fairley (1946) under controlled experimental conditions.

The relapse phenomenon was studied in various strains of *P. vivax* and considered by many to be caused by a cryptic schizogony cycle of the type demonstrated in avian malaria. (Ruge, 1936a, 1936b; James, 1937; Verney, 1938c, 1938d; Kikuth, 1938, 1943; Vich and Rey, 1939; Casini, 1939; Tarsitano and Lucrezi, 1939; Raffaele, 1939, 1941a, 1941b, 1944a, 1944b, 1946a; Bianchi,

1940; Davey, 1944, 1946; Mudrow and Reichenow, 1944; Taliaferro, 1944, 1947; Fairley, 1946a, 1946b, 1947; Shute, 1946; Huff, 1947; v. Haller, 1947.) Some of the Italian school of malariologists, headed by Corradetti and Ascoli, held to the opinion of Bignami (1913) in the matter of relapses (Corradetti, 1943).

Actual descriptions of the cryptic forms, leaning heavily upon the knowledge of avian e-e forms, were forthcoming, but, as a whole, these fell short of the *desiderata* of the more eminent of the malariologists.

There were three groups of parasites involved at the time: (1) the chiropteran, (2) the simian, and (3) the human. The two former groups contained a number of species thought at that time to belong to the genus *Plasmodium* which later were shown to have their true position elsewhere; none the less these parasites will be dealt with in order to give a complete perspective.

Schingareff (1906) had described e-e stages of *Polychromophilus murinus* (in the 1940s thought to be *Plasmodium murinum*) of the fruit bat. Nothing further was heard of chiropteran malaria until Manwell (1946) redescribed the so-called *Plasmodium pteropi* of fruit bats in New Guinea (the exact genus of this parasite is still unknown). Manwell described extracellular unpigmented segmenting forms in the peripheral blood where the only endocellular forms known were the gametocytes. These forms were rare and resembled the e-e stages in avian malaria. He also noted that the number of nuclei varied greatly. The significance of this observation will be referred to later.

In the following year Mer and Goldblum (1947) discovered *P. murinus* in Palestinian bats and, in addition to the well known circulating gametocytes, they observed intense schizogony in reticulum cells and in cells of the granulocyte series in the bone marrow. Schizonts were found also in lesser numbers in the fixed and wandering macrophages in the liver, lung and kidney. This provided the first full description of e-e schizogony in chiropteran malaria but this parasite noncommittedly described by Mer and Goldblum as a Haemosporidian is not a true *Plasmodium*.

On the subject of the simian malarias Schwetz (1938) in a letter noting the discoveries of James and Tate, recalled his description of "blue bodies" in infections of *Hepatocystis kochi* thought then to be a *Plasmodium*. He recorded his belief that these bodies represented the e-e schizogony forms of *H. kochi*. Goldstein (1939) recorded Coggeshall's fruitless search for e-e forms in an unnamed simian malaria. Corradetti (1941e) reported an equally unsuccessful search for the e-e forms of *P. knowlesi*.

Yokogawa (1942a, 1942b, 1942c) reported the finding of e-e forms of *P. knowlesi* and *P.? taiwanensis* (this latter may be a synonym for *P.? semnopitheci*; at all events, neither is a true *Plasmodium*). They were found in the brain and spinal cord. The number of nuclei was small and the site of development was in endothelial cells These observations were corroborated by Kamachi and Oh (1943a, 1943b). All these observations should be discounted. *P.? taiwanensis* is

probably of the family Haemoproteidae. *P. knowlesi* has been shown to undergo schizogony in the liver. Lastly, the state of the knowledge of simian malaria parasites in Japan was in such confusion as to force any observer to regard such findings as the above with the utmost suspicion.

A valuable contribution to the knowledge of the development of the simian malarias came in 1946 when Colonel Mulligan's team, working under the Indian Research Fund Association, made a preliminary report on their progress (Mulligan, 1947). They found that the blood of rhesus monkeys inoculated with the sporozoites of up to 100 mosquitoes infected with *P. cynomolgi* was consistently negative from about 40 minutes to 9 days after infection, as shown by blood transfusion into clean monkeys. During this time it was found that both the skin at the site of inoculation and the spleen were also not infective. No sporozoites were demonstrable in the area of inoculation after one hour. They reported failure to grow sporozoites of *P. cynomolgi* in spleen, brain or liver whether inoculated into cultures or when explants were taken during pre-patency. They concluded that there was no reason to suppose that the mammalian e-e cycle was comparable with the e-e cycle of either the avian or saurian malaria parasites.

Huff (1947), summarizing the situation on tissue forms of malaria parasites, doubted the validity of Schwetz's claim to have found the schizogonic stages of *H. kochi*, as well he might, for later in the same year Garnham (1947) described the true schizogonic stages of *H. kochi.* It was he who changed the genus of the parasite from *Plasmodium* to *Hepatocystes* and later corrected this to *Hepatocystis.* These schizogonic stages occurred as large merocysts of up to 2 mm. in diameter in parenchymal cells of the liver, easily visible there as white or translucent spots occurring on the liver surface. These merocysts had been seen by Levaditi and Schoen in 1932, as has been described. Garnham's observations were confirmed by Hawking and Hunt (1947). It is now believed that the "blue bodies" of Schwetz represented segments of the cytomeres which occur in the merocysts. These segments break loose and circulate in the peripheral system, coming to rest on occasion in the bone-marrow. Such an explanation may also hold true for the observation of similar bodies by Manwell (1946) in *P.? pteropi*, although no merocysts have been found in this species.

The greater proportion of the work devoted to the e-e cycle of the mammalian plasmodia concerned the human malarias and it is in this field that so many speculations and so many claims were made. Firstly it is necessary to deal with the numerous demonstrations of so-called e-e forms of human malaria parasites and the counter arguments raised by these claims.

Raffaele (1937c) was the first to describe unpigmented schizonts of human malaria parasites, which he claimed to represent the e-e cycle. He found two unpigmented schizonts in the bone marrow taken from the sternum of patients infected with sporozoites of *P. vivax.* One of the schizonts was extracellular while the other lay in a R-E cell, and they were found only after long and patient

search. Raffaele also described two uninucleate forms in R-E cells. In the following years Raffaele (1938d, 1940b) described similar findings in sporozoite-induced *P. falciparum* infections. He summarized his findings later and postulated an e-e cycle of human malaria parasites similar to that which he had postulated for the fate and subsequent development of the sporozoite of avian malaria parasites (Raffaele, 1939), while stressing the comparative rarity of the human forms. He also described a similar form in a *P. malariae* infection (Raffaele, 1940b).

Raffaele's findings were confirmed by Tarsitano and Lucrezi (1939), Jerace (1939), Casini (1939), Bianchi (1940), Brug (1940), Paraense and Da Silva (1941), Coudert and Eyquem (1944), Garcia (1945), Oberlé (1945) and Spanedda and Floris (1945).

Taken together, these reports represented descriptions of unpigmented schizonts and trophozoites both extracellular and within endothelial cells. They were found in the bone marrow, brain capillaries and in the lung of patients with pre-patent acute and chronic infections with *P. vivax*, *P. falciparum* and *P. malariae*. Never more than a few were found and then only after long search. In no case were consecutive growing forms discerned, nor did the number of merozoites ever exceed that of the erythrocytic forms. Generally, the number of nuclei was between 1 and 4. These authors all agreed that these forms represented stages of the e-e cycle and they were supported in this view by Piccioli (1941) and Coudert (1945). The latter author noted the appearance of monocytes and normoblasts in the peripheral blood three days after sporozoite infection. He claimed that this was the result of proliferation of the bone marrow cells sensitized by the pre-e development of the parasite in the R-E system.

Other authors were more cautious in their estimation of the significance of these forms. Matilla and Aparicio (1943) described stages similar to those described by Raffaele but reached no conclusions as to their significance. Lanza (1944) also described extracellular unpigmented stages in *P. falciparum*, but stated his belief that they did not represent part of an obligatory cycle of the parasite and that their appearance was purely accidental. Dubin (1947) described forms which he said "suggested e-e forms of *P. vivax*" grown in tissue cultures of explants from the bone marrow of sporozoite-infected patients taken during prepatency. He himself was dubious about the significance of these forms.

Still other authors discounted these findings altogether. Many eminent protozoologists expressed the belief that, in the words of Angelini: "As yet, a reliable morphological documentation of an endohistiocytic development of *Plasmodium* in human malaria does not exist" (Angelini, 1947). Such opinions were echoed by Reichenow (1939), Corradetti (1940h, 1941h, 1943), Davey (1946) and Huff (1947), even Kikuth and Mudrow (1941a) who supported Raffaele in so much else were unable to do other than doubt his findings in human malaria.

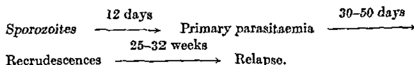
Many negative searches for the cryptic forms were also recorded. Boyd and Kitchen (1939) searched biopsied skin from the site of inoculation of *P. vivax*

sporozoites and found no development in those sporozoites still to be seen in the area after several hours. They concluded that cells of the skin did not represent the destination of the sporozoite.

The search covering 177 bone marrow smears represented by the reports of Quattrin (1941), Parise and Lucrezi (1941), Nucciotti (1942) and Lioia (1942) was consistently negative. These records cover 70 patients infected with *P. vivax*, *P. falciparum* and *P. malariae*, the marrow being taken at the time of pre-patent acute and chronic infection. Fonseca *et al.* (1946) found no e-e forms at any time in the infection in spleen, liver, lung, brain or bone marrow from sporozoite- or blood-induced *P. falciparum* or *P. vivax* malaria cases, nor in bone marrow in sporozoite- or blood-induced *P. malariae* infections, though Fonseca (1944) had earlier reported some e-e-like parasites.

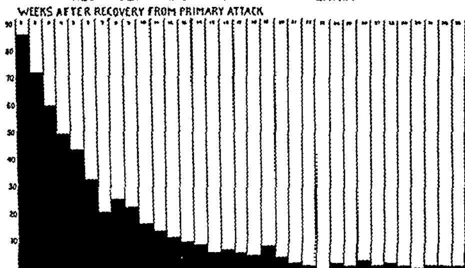
Apart from microscopic data a certain amount of information relevant to the human e-e cycle had accrued. *In the field of chemotherapy it had been shown that quinine and mepacrine were to some degree prophylactic if treatment was* maintained for 11 days after bite in *P. falciparum* malaria. On the other hand, they afforded no protection in *P. vivax* malaria (Ciuca *et al.*, 1937b). Pamaquine was reported to prevent relapses in *P. falciparum* but not in *P. vivax* or *P. malariae* malaria (Peter, 1941). Pamaquine had also been shown to affect the relapse rate of *P. vivax* malaria (Most *et al.*, 1946). All this appeared to indicate some difference between the developmental patterns of *P. falciparum* on the one hand and *P. vivax* and *P. malariae* on the other.

A significant observation had been made by James, Nicol and Shute (1936) on the relapse patterns of *P. falciparum* and *P. vivax* strains used in therapeutic malaria. They noted that whereas untreated *P. vivax* infections always relapsed when sporozoite-induced, this was not necessarily the case in blood-induced *P. vivax* infections. This led them to differentiate between *true relapses and recrudescences of the blood forms. They defined relapses as being parasitological outbreaks at least 8 weeks after the cessation of previous parasitaemia.* Recrudescences *were such rises or apparent outbreaks within this time.* Thus, with the Madagascar strain of *P. vivax* they were able to distinguish a comparatively strict synchronicity of events in sporozoite-induced infections :



In blood-induced infections *no such true relapse occurred.*

As important was their finding that no such pattern existed in sporozoite-induced P. falciparum malaria. The pattern of events in *P. falciparum* malaria *followed* that of blood-induced *P. vivax* malaria in some characteristics, and only recrudescences of parasitaemia from a continual low-grade infection occurred.



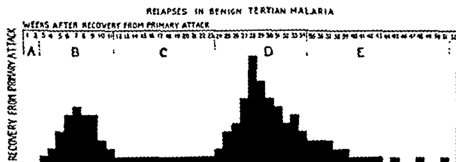


FIG. 1. The two charts display the recrudescence and relapse patterns of *P. falciparum* on the one hand and those of the long term relapse Madagascar strain of *P. vivax* on the other. (From James, S. P., Nicol, W. D. and Shute, P. G., 1936. *Proc. Roy. Soc. Med.*, 29, 879.)

Thus the plot of parasitaemia in sporozoite-induced *P. falciparum* malaria followed Pattern 1 (see fig. 1), whereas sporozoite-induced *P. vivax* malaria showed Pattern 2 (Madagascar strain).

These observations are in a direct line with the observations of Fairley (1946a, 1946b, 1947) with which it is now intended to deal at some length as their importance at the time and today cannot be overestimated.

The development of proguanil (M4888, Paludrine) by the brilliant researches

of the Imperial Chemical Industries workers, Curd, Davey, Rose, Lindquist and others, placed a powerful new tool in the hands of malariologists, and its usefulness in the laboratory was matched by its service in the field. No more capable hands could have been found to wield this new tool than those of Brigadier (now Sir Neil) Hamilton Fairley and, as war would have it, no more ideal conditions could have been found for its use than those existing in Cairns, Australia, in 1944.

Fairley (1946a) carried out controlled trials, using large numbers of Australian Army volunteers to test the efficacy of the new drug and at the same time (Fairley, 1947) to gain more knowledge of the fate of the sporozoite of human malaria. His observations might be briefly summarized here. He found that while proguanil was causally prophylactic in mosquito-borne *P. falciparum* malaria it was only *partially prophylactic in similar P. vivax infections, merely delaying the onset of* fever by 20 days or so. In both cases proguanil was administered at such a time and in such a manner that it could have acted only upon the cryptic pre-e forms and apparently would not be still in effective concentrations in the body at the onset of parasitaemia [i.e. more than 4–5 days prior to parasitaemia (Spinks, 1946)].

Fairley found, by inoculation of massive amounts of blood (250 ml.) into non-immune volunteers from heavily sporozoite-infected volunteers, that the negative phase of the blood was from 1 hour to 144 hours after infection in *P. falciparum* malaria, and from 1 hour to 8 days after infection in *P. vivax* malaria. There was no break in this period, as occurs after 36 hours in some of the avian malarias. From the commencement of parasitaemia in *P. falciparum* malaria until radical cure or recovery, the blood is positive, as shown by inoculation. In *P. vivax* malaria, the blood is negative, as shown by inoculation, after apparent cure or recovery but becomes positive again just before clinical relapse. From these results Fairley concluded that a cryptic pre-e cycle existed in both *P. falciparum* and *P. vivax* malaria, but a subsequent e-e cycle existed only in *P. vivax* malaria. He also postulated 3 generations of 36–40 hours' duration in the pre-e phase of *P. vivax*.

Fairley was able to show that mepacrine and proguanil, like quinine, displayed complete efficacy in blood-induced infections of *P. vivax* malaria and therefore concluded that in human malaria the erythrocytic parasites could not initiate the e-e infection.

Sinton (1946) added the comment that the difference in the action of proguanil upon avian malaria from that shown by Fairley on human malaria must be taken as a warning that work upon avian infections gave no certain indication of the activity of the mammalian infections, a warning which was to be all too often ignored in the following years. This warning was reinforced by Coatney *et al.* (1947) when they showed that sulphadiazine was effective against e-e forms in avian malaria infections but ineffective as a causal prophylactic in *P. vivax* malaria.

Raffaele (1946b), commenting on Fairley's experiments, gave the negative blood phase of Italian strains of *P. vivax* and *P. falciparum* as 4 days for both, comprising, he claimed, two pre-e generations each of 48 hours' duration.

Raffaele (1946b), Davey (1946a) and Huff (1947) all agreed with Fairley that the evidence seemed to point to the lack of any e-e cycle of *P. falciparum* after the pre-e generation. Huff (1947), however, doubted if drug action was a sufficiently constant function to allow conclusions regarding modes of growth of parasites.

A further observation relevant to the existence of e-e forms and their relation to relapses in *P. vivax* malaria was made by Boyd (1947) who showed that the immune responses to *P. vivax* malaria were directed purely against the blood forms and not against sporozoites or such cryptic tissue forms as might exist. Thus he concluded that persistence of an e-e cycle could occur without danger of interference from specific immune reactions.

There remained two phenomena which were difficult to explain by any of the existing theories. These were the twin cases of the long-term relapse and the long-term latency with delayed primary parasitaemia which occurred in the so-called "temperate zone" strains of sporozoite-induced *P. vivax* malaria.* Shute (1946) summarized his experiences with the Madagascar strain of *P. vivax* which displays both of the above phenomena. He laid down 6 conditions under which long-term latency could occur.

1. Natural circumstances.
2. Drug treatment, e.g. proguanil.
3. Mixed infections, e.g. *P. falciparum* and *P. vivax* malaria.
4. Host immunity to another strain of *P. vivax*.
5. Attenuated sporozoites.
6. Too few sporozoites.

Shute found that the delay in primary parasitaemia in such cases generally coincided with the time of long-term relapse in other cases infected at the same time and proceeding normally.

The various explanations put forward for these phenomena will be dealt with later.

* Strains of *P. vivax* showing similar relapse and latency patterns have been described from Madagascar, India and the French Cameroons, thus the term "temperate zone" is misleading, convenient as it may be in providing an explanation of the phenomenon in the terms of hibernation of mosquitoes. However, it is my personal opinion that *P. vivax* is fundamentally a parasite of temperate climates and that its appearance in tropical zones represents a comparatively recent invasion of these areas. The appearance of the distinct South-West Pacific strains can be explained by the same genetical reasoning *as has been used in the case of the marsupials of Australasia.* This presupposes the selection of the long-term relapse strains in temperate areas sometime after the geographical separation of Australasia and that the South-West Pacific strains of *P. vivax* indicate the true ancestral nature of *P. vivax* development.

CHAPTER 4

THE PRESENT STATE OF THE KNOWLEDGE OF THE EXO-ERYTHROCYTIC CYCLE OF THE AVIAN AND SAURIAN PLASMODIA

(*a*) SPECIES: MORPHOLOGY AND CHARACTERISTICS

We have, at the present time, some knowledge of the e-e cycle of 12 of the avian plasmodia and of 4 of the saurian plasmodia.

These are:

Avian	*P. gallinaceum*	*P. juxtanucleare*
	P. relictum	*P. durae*
	P. cathemerium	*P. hexamerium*
	P. lophurae	*P. elongatum*
	P. fallax	*P. vaughani*
	P. circumflexum	*P. huffi*
Saurian	*P. mexicanum*	*P. agamae*
	P. pitmani	*P. lacertiliae*

Only in the first 5 plasmodia of this list is our morphological knowledge in any sense complete in that only of these 5 is the pre-e development known. It is intended to give a detailed account of the development of *P. gallinaceum* in the fixed tissue cells of the young domestic chick from the sporozoite and erythrocytic trophozoite to the phanerozoite. Descriptions of other species will be discussed later in relation to this account and therefore less fully.

Morphology and characteristics

Plasmodium gallinaceum Brumpt, 1935

1. *Pre-erythrocytic cycle.* The pre-e cycle of *P. gallinaceum* was fully detailed by the classic work of Huff and Coulston (1944) and Coulston, Cantrell and Huff (1945). These workers found that the sporozoite, when injected into the skin of a chick either by syringe or by bite of an infected *Aëdes aegypti*, is no longer demonstrable in the blood after 25 minutes. They were able to show that one or more sporozoites enter a cell of the lymphoid-macrophage system after some time spent in the intercellular spaces. *After 6 hours those sporozoites which have not gained* harbourage in a cell disappear. Those sporozoites in heterophil granulocytes appear to have been actively ingested and are apparently dead or dying as a result of phagocytic action. It is not known whether those which survive in cells

take any active part in entering the cell. Six hours after inoculation the intracellular sporozoite commences to lose its spindle shape and becomes a rounded cryptozoic trophozoite of about 2μ in diameter. There is little change after the spherical shape has been achieved until about 18 hours after inoculation. Some vacuoles occasionally appear. From 18 to 24 hours after inoculation division of the single nucleus begins and binucleate cryptozoic schizonts appear. There is a concomitant increase in size. From 24 to 36 hours growth is rapid and nuclear division active. The organism is still spherical or slightly ovoid and contains up to 32 nuclei by 36 hours. From this time onwards, up to about 48 hours, mature schizonts appear and their host cells rupture. The mature schizont measures some 6–10μ and may contain from 32 up to 200 nuclei, depending on the time of development and the number of nuclear divisions thereby allowed.

The mature schizont consists of elongate merozoites about 2μ in length, frequently arranged regularly about a central core with one end appearing to rest upon the core. Some suspicion of pseudo-cytomere formation may be seen and the schizont may bear in miniature some superficial resemblance to one of its forebears—the oöcyst at the sporozoite formation stage. All merozoites of this first cryptozoic generation have been released by 48 hours.

The merozoites released from this first generation apparently all re-enter lymphoid-macrophage cells, largely at the site of inoculation. Some merozoites are borne by the blood stream all over the body and are demonstrable in the blood stream by inoculation into clean birds. Once more multiple invasion of cells occurs. Theoretically it cannot be said that erythrocytes are not invaded at this stage. Inoculation experiments are not conclusive on this point. *P. cathemerium* has been shown to infect the peripheral blood from 24 hours onwards after sporozoite inoculation (Kikuth and Mudrow, 1938a). It can be argued, however, that, in this parasite especially, this effect may be the result of invasion of the wandering macrophage cells of the blood. In the case of *P. gallinaceum*, Coulston, Cantrell and Huff (1945) and Curd, Davey and Rose (1945) have shown that though the blood becomes infective 30 hours after sporozoite inoculation, it ceases to be so by 48 hours and remains non-infective until 79 hours after sporozoite inoculation. It would appear then in practice that the circulating cryptozoic merozoites cause the blood to become infective from 36 to 48 hours after sporozoite inoculation, but after this period all merozoites invade fixed tissue cells or die, thus becoming unavailable for blood inoculation.

The cryptozoic merozoites, on entering the lymphoid-macrophage cells, repeat exactly the cryptozoic generation except in so far as the resultant merozoites are held by some to be of two types, one type larger, the other smaller. This new brood is known as the metacryptozoic generation. The distribution of metacryptozoites is more general than that of the sporozoite-induced cryptozoites The cryptozoic merozoites may be carried by the blood to all organs of the host, which contain the type of cell necessary to the growth of the parasite. This

type of cell appears to be the reticulum cells, particularly of the spleen. Some organs are unsuitable for the growth of the parasite owing either to lack of suitable cells or to relative inaccessibility (e.g. brain or bone marrow). The metacryptozoic schizonts, which resemble their cryptozoic forebears, in turn produce and release elongate merozoites, some of which re-invade fixed tissue cells of perhaps a slightly wider range than previously. Other metacryptozoic merozoites invade the circulating erythrocytes and reticulocytes, thus initiating the blood cycle. From this time (72–80 hours) onwards the blood is consistently infective.

This sequence of events has been confirmed wholly or partially by Huff and Coulston (1946), Shortt and Malamos (1947), Gordon and Hancox (1947) and Huff (1951). It has been found to hold true in the chorio-allantois of chick embryos by Fonesca *et al.* (1946) and McGhee (1949b).

The German workers Reichenow and Mudrow-Reichenow believed that the destination of the merozoites of the metacryptozoic and subsequent generations can be determined by the study of their shape and size (Reichenow and Mudrow, 1943; Mudrow and Reichenow, 1944; Reichenow, 1947; Mudrow-Reichenow and Reichenow, 1949; Mudrow-Reichenow, 1952). They, in common with other workers (Huff and Coulston, 1944; Hawking, 1945, 1951; Coulston and Huff, 1947; Tonkin and Hawking, 1947; McGhee, 1949b; Huff *et al.*, 1950; Inoki, 1951; Herbig-Sandreuter, 1953) distinguish between two different types of merozoites produced by the e-e schizogony in birds. One type is larger, elongate with abundant cytoplasm and measures some 1·5–2·5μ. These large merozoites have been designated macromerozoites and their progenitor the macroschizont. The other type is smaller, spherical and measures 1–1·5μ in diameter. These have been designated micromerozoites formed by microschizonts and consist largely of chromatin. Some 100–200 macromerozoites form a macroschizont whereas 500–1000 micromerozoites form a microschizont. The German workers postulated that macromerozoites are destined to invade tissue cells whereas micromerozoites find harbour in red blood cells.

This theory of dimorphic schizogony has recently been seriously challenged by one of its former advocates. Despite the occurrence of dimorphic schizogony in *Leucocytozoön* and some of the haemogregarines, Huff (1952) decided that this problem required further study. He carefully measured the diameters of metacryptozoic merozoites and found that they represented a steady gradient from 1·0μ to 2·5μ with no appreciable break in between to warrant a differentiation into two sizes. This study would appear to remove the morphological bases from the theory of dimorphism and, convenient as it was, the present author feels that it should be discarded in its present form unless new information comes to light. Its theoretical basis, however, should be retained. This basis is Raffaele's contention that e-e schizogony results in haemotropic and histiotropic merozoites, and that they arise from schizonts also so predestined rather than that both types arise from a single heterotrophic schizont (Raffaele, 1938a, 1938b).

The above results have been obtained by means of exceptionally heavy sporozoite dosages (100–200 heavily infected *Aëdes*), and in this lies the secret of success in the work dealing with the pre-e cycle. At the same time this technique has obtained results not entirely consistent with the events as they occur in nature where infection is usually the result of a relatively small number of sporozoites from the single bite of a mosquito. In such a case it would be expected that the majority of cryptozoic and metacryptozoic schizonts would be localized at the site of bite and that only a very few metacryptozoites would appear elsewhere. Under these circumstances the erythrocytic forms would not become microscopically demonstrable until after some 4, 5 or 6 generations of a seemingly pre-e cycle and representing a time lapse of 7 to 11 days rather than the theoretical 3 days. At all events, the end of the metacryptozoic generation ends what is accepted as the true pre-e generations.

2. *Exo-erythrocytic cycle.* The e-e generations, known as phanerozoites, may arise from the metacryptozoic merozoites in the same manner as has been described above after sporozoite infection. They may arise also from erythrocytic forms after blood infection. It is presumed that they may be reinforced also from the blood phase which follows from sporozoite infection. This last point has not been conclusively proved but it may be taken without serious demur. Lastly, phanerozoites may arise from infection by phanerozoites without the interpolation of erythrocytic forms. *Thus, phanerozoites will arise no matter what is the mode* of infection, provided that the host animal has no immunity to this form of the parasite.

Four features distinguish to a greater or lesser degree the phanerozoite from the cryptozoite or metacryptozoite:

(*a*) Owing to their relative ubiquity, the phanerozoites spread all over the body of the host whereas cryptozoites and, to a lesser degree, metacryptozoites tend to be confined to the site of inoculation of sporozoites. Further, the type of cell parasitized by phanerozoites displays a wider range of choice. There is a progressive tendency to invade vascular endothelium in preference to reticulum cells. The lung and the brain tend to become the favourite sites for invasion of vascular endothelium. In such cells the phanerozoites take up more elongate shapes as dictated by the shape and width of the capillary involved. Some phanerozoites may be twisted and branching owing to these outside influences (*cf* *Haemoproteus*, p. 82).

(*b*) The phanerozoites tend to show the so-called microschizogony to a marked degree. The majority of phanerozoic schizonts contain 500–1000 merozoites of a smaller mean size when found in vascular endothelium, particularly in the brain. Cryptozoic and metacryptozoic schizonts normally contain some 100–200 merozoites of a larger mean size (Huff, 1952). The reason for this phenomenon, which led the German workers to postulate the theory of dimorphism, remains obscure. Huff (1952) suggests that it is dictated by the shift from reticu-

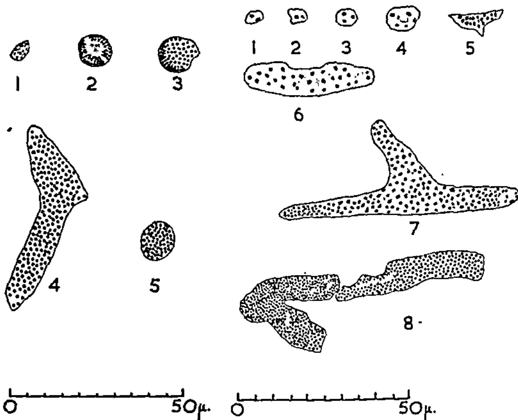


FIG. 2. FIG. 3.

FIG. 2. Phanerozoites of *P. gallinaceum* in section. Stained with Giemsa-colophonium.

1. Immature schizont in a R-E cell of the liver. 2. Mature schizont in a R-E cell of the liver.

Note the circular hollow nuclei and the tendency to elongation of the merozoites and meroblastic formation. 3. Mature schizont in a R-E cell of the liver. 4. Mature schizont in a vascular endothelial cell of the brain in longitudinal section. 5. Mature schizont in a vascular endothelial cell of the brain in transverse section.

FIG. 3. Phanerozoites of *P. gallinaceum* in brain smears. Stained with Giemsa.

1. 2-nucleate schizont. 2. 2-nucleate schizo[illegible] [illegible]ucleate schizont. 4. *6-nucleate schizont with vacuole.* 5. Immatu[illegible] [illegible] Immature schizont. 7. Immature schizont branching along two c[illegible]

lum host cells to vascular endothelial host cells, the latter allowing a more profuse development. Corradetti (1938a, 1938b) suggested that the quality and quantity of available nutriment may be concerned *in the size of e-e forms and that this may* also play its part. The present author would like to add that, in his opinion, immunity may play its part as well. It may be that immune reactions cause the shift and at the same time dictate the production of larger numbers of smaller merozoites.

(c) Under these circumstances the merozoites themselves tend to lose their pronounced elongate appearance when found in vascular endothelial cells. They become smaller and spherical. In the early generations circular merozoites are taken by Huff (1952) to be a sign of degeneration, whereas later they may be taken as a sign of progressive infection.

(*d*) Finally, although the size of a phanerozoite found *in the reticulo-endothelium* may roughly correspond to the size of pre-e schizont, i.e. up to *12μ*, the phanerozoites may grow to far greater sizes in vascular endothelium. (See figs. 2 and 3.)

The phanerozoites can be found *all over the body of the host regardless of the* mode of infection. They have been demonstrated in the fixed endothelial cells in the brain, lung, liver and spleen (James and Tate, 1938) in the muscle and thymus (Coulston, Cantrell and Huff, 1947) in the heart, intestine, pancreas, fat, bone marrow, striated muscle and kidneys (Tullis, 1947) in the pia mater (Lewart, 1948a) in the spinal cord, supra-renals, testis, ovary and lymph glands (Inoki, 1951b). Further, they have been found in all erythrocyte precursors (Zuckerman, 1946), in wandering monocytes and endothelial cells (Mosna, 1947) and in lymphocytes, basophil and polychromatophil erythroblasts and heterophils in particular (McGhee, 1949a). Phanerozoites can arise in a strain of *P. gallinaceum* derived from a single erythrocytic parasite (Downs, 1947).

The patterns of e-e schizogony of *P. gallinaceum* which may arise from various unnatural modes of infection are interesting and will be detailed here. Four main types of e-e schizogony can be distinguished when various hosts or inoculation techniques are employed. They are .

Type A. Normal natural e-e schizogony.
Type B. Early acute e-e schizogony in the absence of erythrocytic schizogony.
Type C. Late acute e-e schizogony following acute erythrocytic schizogony.
Type D. *P. elongatum* type of e-e schizogony in erythrocyte precursors as well as normal e-e schizogony.

Type A

This is the normal subacute development of e-e schizogony as seen in consecutive chick-mosquito passage of *P. gallinaceum* or presumably in nature. The phanerozoites appear in moderate numbers only. *The incidence of e-e schizogony*

is concomitant with erythrocytic schizogony and the apparent pre-patency of both forms is coincident at 4–14 days, the time being in inverse proportion to the number of sporozoites inoculated (Coatney, Cooper and Miles, 1945; Coatney, Cooper and Trembley, 1945a, 1945b; Haas *et al.*, 1948). Both e-e and erythrocytic forms appear in only relatively low numbers and both disappear after about 15 or 16 days. (Haas *et al.*, 1948; Greenberg *et al.*, 1950a.) This type of development remains largely unaltered by passage via emulsions of brain containing phanerozoites but is altered to Type C by continuous blood passage. However, this latter characteristic is not " fixed " as a brain emulsion transfer will cause a reversion to Type A after as many as 53 blood transfers (Greenberg *et al.*, 1950a). Greenberg and his co-workers considered therefore that the genetic mutation to the " fixed " Type C was a rare phenomenon. The rarity of the phenomenon would be enhanced by the relatively low numbers of parasites available for the first sub-passage from Type A, such a low inoculum obviously containing few, if any, mutants.

Type A e-e schizogony can apparently be produced by the inoculation of cryptozoites and metacryptozoites from tissue cultures (Laird *et al.*, 1950; Dubin, 1952). Dubin considered that this argued some weakening in the virulence of the pre-e forms through tissue culture as phanerozoites from tissue culture produce Type B schizogony. In fact a special explanation is probably unnecessary to cover this point. It should be remembered that Type B schizogony is produced by inoculated phanerozoites from tissue culture when such phanerozoites were produced from a " fixed " Type C e-e schizogony (Lewert, 1950). Should the phanerozoites have been produced from a Type A schizogony then they in turn would produce a Type A schizogony as above despite the intervention of cultivation. It may be assumed therefore that the sporozoites used by Dubin were produced by a strain showing Type A characteristics whether obvious or suppressed as in the experiments of Greenberg and his colleagues, and therefore Dubin's pre-e forms carried with them the characteristics of Type A. This then amounts to the same phenomenon as was demonstrated by Greenberg *et al.* (1950a) and tissue culture as such is irrelevant.

Type B

This type of e-e schizogony is characterized by an early acutely pathogenic proliferation of e-e forms in the absence of erythrocytic schizogony until a day or two before death due to the e-e forms. It can be achieved by the sporozoite passage of a strain previously blood-passaged for some time and presumably " fixed " as a Type C infection. The Type B infection has been reported without particular note being taken of it by Ungo-Mugdan (1939), James (1939), Zain (1941b), Fonesca *et al.* (1946) and Paraense (1946, 1947a). The type was first noted in detail by Haas and his colleagues when controlled experiments were used to determine the various patterns of e-e development in *P. gallinaceum* infections (Haas *et al.*, 1946; Haas *et al.*, 1948). Trembley, Greenberg and Coatney (1951a)

have also noted this type of e-e schizogony after sporozoite transfer of a Type C infection, as has the present author.

The Type B infection can also be obtained by infection with phanerozoites derived from Type B or "fixed" Type C infections though not from Type A infections or "unfixed" Type C infections derived from Type A infections. Such phanerozoites are usually contained in emulsions of brain as being the organ containing and carrying over the maximum number of phanerozoites and the minimum number of erythrocytic forms (Mosna, 1940; Adler and Tchernomoretz, 1941; Zain, 1941b; Corradetti, 1942b; Haas *et al.*, 1945; Haas *et al.*, 1946; Zuckerman, 1946; Fonesca *et al.*, 1946; Haas *et al.*, 1948; Verolini, 1949; Greenberg *et al*, 1950a; Inoki, 1951b; Cavaceppi, 1951; Corradetti and Cavaceppi, 1952; Richeson *et al.*, 1953 and personal observation). Such phanerozoites may also be derived from the cultivation *in vitro* of the above types of e-e schizogony and when inoculated usually produce Type B infections (Lewert, 1948b, 1950a, 1950b; Meyer 1949a). However, phanerozoites produced in tissue cultures of spleen from chicks infected by sporozoites of an unidentified strain are known not to produce Type B infections. This is the case at the laboratories of the Wellcome Foundation, London, where phanerozoites produced *in vitro* are producing on inoculation into chicks what appears to be a Type A e-e schizogony pattern. The strain of *P. gallinaceum* has been passaged so variously that it is impossible to designate it according to any of the types and it seems probable that it has retained the basic Type A characteristics as its fundamental pattern through its many vicissitudes. Thus, on phanerozoite passage it produces a Type A e-e pattern (Vincent, private communication). Somewhat similar results have been recorded by Lewert (1950b).

Cavaceppi (1951) maintains that Type B e-e schizogony ceases after 25 or so days. Splenectomy has no effect upon the Type B pattern when induced by phanerozoites (Verolini, 1949; Corradetti and Cavaceppi, 1952). Inoki (1951b) has claimed that *continuous brain emulsion passage of Type B results in a localization* of e-e schizogony in the brain. Other workers have not confirmed this observation. Type B infections are also produced by the blood passage of Type C infections at the chronic stage, that is, during the late e-e schizogony stage characteristic of the Type C infection (Mosna, 1940; Zain, 1941c; Corradetti, 1942b). The erythrocytic infection is usually latent at this stage, which makes this last observation the more interesting as it would appear to denote either a number of e-e forms circulating in wandering macrophages or an exclusive histiotropism in the merozoites of the few erythrocytic forms carried over.

Occasionally a chick or chick embryo in a Type A infection series will display a Type B infection (Haas *et al.*, 1948).

Type B pattern is not a "fixed" characteristic of the strain displaying it. Continuous blood passages in chicks (>15) or even brain emulsion passages through fowls will cause it to revert to Type C (Lewert, 1948b). Continuous

sporozoite passage will cause it to revert to Type A (Haas *et al.*, 1948; Lewert, 1950b).

TYPE C

This type has received more attention than other types and is the subject of a host of observations. It is typified by an early appearance of an acutely virulent blood phase which frequently kills the chick host in 5 to 10 days. If the animal survives, as do older birds or turkey poults or if schizontocides are used, the e-e forms appear late in the infection, from about 11 to 25 days after inoculation. This late e-e phase frequently kills the animal, particularly when schizontocides have been used. Type C pattern is produced by continuous blood passage of any type of *P. gallinaceum* infection in chicks or turkey poults so long as the primary parasitaemia is survived. (Corradetti, 1938b, 1938c, 1940a, 1940c, 1940d, 1940g, 1940e, 1940f, 1941b, 1941c, 1941d, 1942a, 1942b, 1942c, 1943; Ungo-Mugdan, 1939; James, 1939; Jacobi, 1939; Ritis, 1940; Mosna, 1940; Launoy, 1940; Zain, 1941b; Adler and Tchernomoretz, 1941; Paraense, 1946, 1947a; Gramiccia and Saccà, 1947; Tullis, 1947; Haas *et al.*, 1948; Verolini, 1949; Lewert, 1948a, 1950b; Greenberg *et al.*, 1950a; Trembley, Greenberg and Coatney, 1951c; Corradetti and Cavaceppi, 1952; Richeson *et al.*, 1953, and personal observation.) The e-e forms have been found as early as 4 days after blood inoculation (Tullis, 1947) and I have seen them after 7 days but they are not found in any numbers until about 11 days after infection. Much of the observation has been conducted upon the brain which is not the most suitable organ in which to search for early e-e forms owing to the late switch from R-E to vascular endothelium referred to above. Thus, as late as 1952 Corradetti and Cavaceppi insist that no e-e forms are found in blood-induced infections until at least 8 days after inoculation when the brain is used as the site for research. Tullis (1947) on the other hand was able to demonstrate reasonable numbers of e-e forms in the spleen, heart and lung on the 6th and 7th days after blood inoculation, the infection being Type C. However, Type C is characterized by a large increase of e-e forms at about the 11th or 12th day after infection for reasons which remain obscure despite the work of Corradetti.

Gramiccia and Saccà (1947) have suggested that the 11 days represent the time taken for the parasite to regain its normal characteristics (i.e. Type A) after the unnatural method of transfer. This does not explain the apparent curtailment of this time when schizontocides are used to suppress the erythrocytic development and when the size of the inoculum is probably of some importance (Adler and Tchernomoretz, 1941). As the genesis of these late e-e stages will be taken up in the section dealing with immunity, the views of Corradetti and others will not be discussed here. Corradetti has frequently stated that the e-e schizogony in this pattern ceases from 25 to 30 days after inoculation. This, undoubtedly, is generally true, despite a report of e-e forms 37 days after inoculation (Jacobi, 1939).

It should be stressed at this point that these figures apply only to the Type C infections and that such infections are the most artificial possible as no mosquito transfers are involved.

Paraense (1946, 1947a) has shown that the Type C e-e forms are highly virulent and cause death when erythrocytic schizogony has been suppressed by quinine. Death is by blockage of the brain capillaries. Haas *et al.* (1948) and Lewert (1948b and 1950b) have shown the blood passage of Type B results in Type C. Trembley, Greenberg and Coatney (1951c) have shown that blood-passage of a strain displaying no e-e schizogony results in a Type C infection with late e-e schizogony. Haas *et al.* (1948) have shown that blood-passage of Type D results in Type C infections. Greenberg, Trembley and Coatney (1950a) have shown that their Type C pattern strain is "fixed" and will not regain its parent Type A pattern on sporozoite passage which merely results in the "unfixed" Type B pattern. They conclude that this argues fixed characteristics, one of which is the inability of phanerozoites of Type C to produce merozoites capable of infecting erythrocytes on passage. This conclusion supports the theory of haemotropism and histiotropism of phanerozoic merozoites. Verolini (1949) has shown that splenectomy fails to affect the course of the Type C e-e schizogony.

TYPE D

This type is somewhat obscure as yet and has been brought into being by the present author to cover certain variable phenomena. However, these phenomena are of such systematic importance that it has been felt they deserve special grouping. *P. gallinaceum* is well known to parasitize lymphoid-macrophage cells as it does in the above three types of infection. It is not known normally to infect erythrocyte precursors as does *P. elongatum*.

Zuckerman (1946) infected chick embryos by placing e-e forms in macerated brain on the chorio-allantois. She noted that in one embryo the resultant e-e forms appeared in wandering leucocytes, lymphocytes, thrombocytes and all of the erythrocyte precursors. Only in erythrocytes and mature polychromatophil erythroblasts was pigment produced. The number of merozoites produced was only 15 or so. At the same time normal *P. gallinaceum* e-e forms were seen in the brain of the same embryo. Haas and his colleagues had described non-pigmented parasites in the circulating blood of chick embryos infected with phanerozoites but had assumed that phanerozoite-induced merozoites had temporarily lost the power to produce pigment (Haas and Ewing, 1945; Haas, Feldman and Ewing, 1945; Haas, Wilcox and Ewing, 1945).

McGhee (1949a, 1949b) infected chick and duck embryos with sporozoites of *P. gallinaceum* and noted in both hosts that some of the younger embryos displayed e-e infections in the heterophils, lymphocytes, basophil and polychromatophil erythroblasts as well as in more normal sites. Some pre-e forms were seen in heterophils. McGhee's (1949c) observations on *P. lophurae* were even more con-

clusive. These will be referred to later. From these results it would appear that Type D is characterized by the appearance of phanerozoites in the circulating blood, particularly in erythrocyte precursors as well as in normal sites. It can be produced by the inoculation of sporozoites or phanerozoites into young (>9 days old) embryos.

Various hosts besides the domestic fowl have been shown to harbour e-e forms of *P. gallinaceum*. Huff and Coulston (1946) have demonstrated pre-e forms in geese and ducks, and Huff (1951) has demonstrated pre-e forms in turkey poults, pheasants and canaries. The pre-e development in ducks was somewhat abnormal. The phanerozoites have been shown to occur in turkey poults after blood inoculation and duck embryos after sporozoite inoculation (Corradetti, 1942b; McGhee, 1949a).

Gramiccia and Saccà (1947) showed that the younger the chick host the larger the number of e-e forms which appeared but, on the other hand, no alteration of time of appearance was noted. Herbig-Sandreuter (1953) demonstrated that high altitudes (10,500 feet) retarded the time of appearance of e-e forms in birds infected at ground level. Varied reports have been made upon the comparative density of e-e forms in the various organs or cells. When the available material is collated, it seems that the organs most consistently and heavily infected are the lung, spleen and liver and possibly the heart and kidney. The brain has long been the favourite site for search but this is due more to the ease of demonstration of e-e schizogony for technical reasons than to any greater proliferation at this site. Among the less frequently invaded organs are the bone marrow, pancreas, intestine, suprarenals, testis, etc. Least often invaded are the circulating leucocytes, lymphocytes, plasma cells. My own findings, with impression smears of organs from a phanerozoite-induced infection in an 8-day-old chick 7 days after infection, show the following numbers of schizonts per 100 fields, with 5× ocular and $\frac{1}{7}$ objective:

Lung	61 schizonts	Brain	17 schizonts
Spleen	42	Intestine	7
Liver	40	Pancreas	6
Heart	27	Bone marrow	1
Kidney	25		

and one form seen in a monocytic leucocyte after considerable search. These figures must be regarded with caution as the amount of tissue searched in each case probably varied considerably owing to variations in impression smears from organ to organ and slide to slide. No doubt the numbers in the brain increase with the duration of the infection owing to the progressive switch to vascular endothelium.

*Plasmodium relictum** (Grassi and Feletti, 1891)

The pre-e generations of *P. relictum* were the first of the pre-e cycles of plasmodia to be fully described. The full cycle was demonstrated in canaries by Reichenow and Mudrow (1943) and Mudrow and Reichenow (1944). Pre-e forms had been noted previously by Mudrow (1942a, 1942b). This work was confirmed by Huff and Coulston (1946), Coulston and Huff (1947) and Huff (1948d, 1951). The timing of the cryptozoic and metacryptozoic generations, and the nature of the host cells, were found to coincide with *P. gallinaceum* despite the exceptionally short pre-patent period of 65 hours demonstrated by Raffaele (1936b, 1936c) and Missiroli (1937). There is one major difference between *P. relictum* and *P. gallinaceum* pre-e forms. The *P. relictum* cryptozoic and metacryptozoic merozoites are spherical and number some 30 to 40 as compared with the 100 or so elongate merozoites of *P. gallinaceum*. The German workers noted some late developing forms of *P. relictum* containing 100 or more merozoites, but these are presumably equivalent to the same forms in *P. gallinaceum* infections producing 200 or more merozoites. Owing to this difference, the overall size of the *P. relictum* pre-e schizonts is a little smaller than the equivalent forms of *P. gallinaceum*. No workers have confirmed the contentions of Missiroli concerning the development of the sporozoite of this species. All workers observed the so-called macro- and microschizogony.

The German workers have shown in their illustrations of the pre-e forms a rather unusual form of nuclear structure. This structure, so far described only for *P. relictum*, shows the nucleus to be made up of a group of 4 or more nucleoli, aggregations of chromatin or chromosomes thus: No comment is made as to the significance of this structure and speculation as to its nature is at present useless. The present author has been unable to detect similar nuclear structures in phanerozoites of *P. relictum*, although structures somewhat similar have been seen in growing *Haemoproteus* schizonts and growing *Nycteria* schizonts. This observation was not confirmed by Coulston and Huff (1947) using Maximow stain, which, however, they admit is a poor stain for the nuclei of malaria parasites. The present author is inclined to reject this discrete particle structure for various reasons:

1. The method of dry fixation and Giemsa stain used by Mudrow and Reichenow is not conducive to fine nuclear staining or even the retention of original nuclear structure.
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3. The present author's experience with wet fixation and Giemsa or Feulgen staining of *P. relictum* and *P. gallinaceum* phanerozoites has revealed in general only a typical spherical nucleus as is illustrated in fig. 2, although, admittedly, at some stages the nucleus may be more square.

The phanerozoic generations of *P. relictum* follow largely the same pattern and distribution as those of *P. gallinaceum* although they have not been so exhaustively studied. Phanerozoites were first seen by Raffaele (1936b) and Kikuth (1937a). Kikuth described forms appearing 72 hours after sporozoite inoculation and it may be that these were metacryptozoites. Type C pattern has been demonstrated (Bishop *et al.*, 1947; Sergent, 1949a). Type B pattern may be assumed to occur from the work of Rodhain (1938a). Type D pattern occurs in chronic infections in adult canaries on blood inoculation (Sergent, 1949a).

The phanerozoites are a little more vacuolated than those of *P. gallinaceum* (Manwell, 1940). The phanerozoic generations persist much longer (up to 2½ years) in blood-induced infections than do those of *P. gallinaceum* (Sergent, 1949a). In the laboratory phanerozoites have been demonstrated, after sporozoite infection of canaries, by Rodhain (1938a, 1938b), Manwell (1940), Reichenow and Mudrow (1943), Mudrow and Reichenow (1944), Huff and Coulston (1946), Coulston and Huff (1947) and Huff (1948d). They have been demonstrated in blood-induced infections by Hegner and Wolfson (1938a), Rodhain (1938a, 1939), Manwell (1940), Dobler (1941), Corradetti (1941g), Bishop *et al.* (1947), Huff (1948d), Redmond and Fincher (1949), Sergent (1949a), and Inoki (1951b). Some strains of *P. relictum* have been shown by Hegner and Wolfson (1938b) to produce no phanerozoites on blood inoculation. Later, Dobler (1941) showed that one of these strains produced phanerozoites in a more suitable host.

In hosts other than canaries Huff and Coulston (1946), Coulston and Huff (1947) and Huff (1948d, 1951) have demonstrated pre-e forms in pigeons, two species of doves, turkey poults and pheasants. Huff and Coulston (1946) and Fincher and Redmond (1949) have demonstrated phanerozoites in pigeons. In nature, phanerozoites have been described in various wild-caught passerines (Taddia, 1938; Taddia and Viero, 1940; Beltran, 1944; Jacobs and Shortt, 1951, and personal experience). Penguins in the Antwerp Zoological gardens and in nature have been demonstrated to harbour phanerozoites by Rodhain (1938a, 1939) and Fantham and Porter (1944). Wenyon (1948) thought that this *Plasmodium* of penguins might be a new species but it has been generally accepted as a variation of *P. relictum*. One feature noted by Redmond and Fincher (1949) is interesting. They demonstrated that a proguanil-resistant strain of *P. relictum* produced a greater number of phanerozoites than the parent sensitive strain.

Plasmodium cathemerium Hartman, 1927

The pre-e forms of *P. cathemerium* were first demonstrated by Kikuth and Mudrow (1938b, 1939a) and the entire pre-e cycle was demonstrated by Reichenow

(1947), Mudrow-Reichenow and Reichenow (1949) and Huff (1951). The pre-e generations closely resemble those of *P. relictum*. In their earlier work the German workers believed that only one generation occurred between sporozoites and the first erythrocytic forms (Kikuth and Mudrow, 1939a; Mudrow, 1940; Mudrow, 1942a, 1942b), but they were to correct this view later. The pre-patent period has been shown to be 72 hours by Warren and Coggeshall (1937).

Phanerozoites were demonstrated in canaries after sporozoite inoculation by Kikuth (1937b), Kikuth and Mudrow (1937, 1938a, 1938b, 1939b, 1939c, 1941b), Mudrow (1940), Porter (1942), Schuleman (1942), Reichenow (1947) and Mudrow-Reichenow and Reichenow (1949). Phanerozoites were demonstrated in canaries after blood inoculation by Kikuth and Mudrow (1937, 1939c), Kikuth (1937a), Hegner and Wolfson (1938a, 1938b, 1939), Corradetti (1938a, 1938b, 1941h), Hewitt (1940a), Wolfson (1940a) and Inoki (1951b).

Phanerozoites have also been demonstrated after phanerozoite inoculation by Kikuth and Mudrow (1939c) who noted also that Type B and Type C patterns occur in this parasite. The phanerozoites themselves closely resemble those of *P. relictum*.

There is one major difference in distribution reported by Kikuth (1937b), Kikuth and Mudrow (1938b, 1939c) and Corradetti (1938a, 1938b). These authors have described e-e forms in mononuclear leucocytes, pseudo-eosinophils and wandering macrophages of the peripheral blood to a degree considerably more pronounced than in the two previous parasites. This phenomenon was not noted in a special study by Porter (1942). It is unlikely that these workers were studying *Atoxoplasma*, which Hewitt (1940a) has shown to be associated with *P. cathemerium* in some infections, as Kikuth himself drew attention to intra-leucocytic non-plasmodial parasites (obviously *Atoxoplasma*) in his own infected birds. The present author believes, on the available evidence, that this phenomenon *does in fact occur but that too much significance should not be attached to* it as a specific characteristic in the light of the similar development in embryos with *P. gallinaceum* or in chronic infections of *P. relictum*.

Hegner and Wolfson (1938b) have reported a strain of *P. cathemerium* which did not produce phanerozoites on blood inoculation, but Porter (1942) showed that the same strain produced e-e forms on sporozoite inoculation. Wolfson (1940a) showed that a strain of *P. cathemerium* temporarily lost its ability to produce e-e forms on blood inoculation into canaries after passage through ducks and pigeons.

Phanerozoites have been demonstrated exclusively in canaries, but cryptozoites and metacryptozoites have been demonstrated in ducks by Huff (1951).

Plasmodium lophurae Coggeshall, 1938

The pre-cycle of *P. lophurae* has been demonstrated by Huff, Coulston, Laird and Porter (1947) in turkeys, chickens, ducks and guinea-fowl. The cryptozoites

and metacryptozoites more closely resemble *P. gallinaceum* than *P. relictum* or *P. cathemerium*. Only immature forms were seen in heterophils and the general cellular distribution is similar to that of *P. gallinaceum*. The duration of pre-e schizogony was shown to be 60 hours by blood inoculation, other than this the authors were unwilling to commit themselves upon the timing of the generations on the morphological evidence available. However, there seems no reason to doubt that two generations are involved as for the previous parasites. Turkeys proved to be the most satisfactory host to the pre-e forms.

Although Terzian (1941) failed to demonstrate phanerozoites in chicks, Tonkin and Hawking (1947) were successful in obtaining phanerozoites in sporozoite-infected turkey poults. Phanerozoites closely resembling those of *P. gallinaceum* were described as numerous in brain, heart, kidney glomeruli and adrenal cortex, and scanty in spleen, lung, kidney tubule capillaries and sympathetic ganglia. None were reported in the liver or bone marrow. Becker and Manresa (1950) and Manresa (1953) have described phanerozoites in the brain of blood-infected turkey poults.

Wolfson (1940b) failed to find phanerozoites in blood-infected duck embryos but McGhee (1949c) demonstrated e-e forms in blood-inoculated chick embryos. McGhee found e-e forms in all forms of erythrocyte precursors. He noted that primitive red blood cells form 9 per cent. of the circulating blood elements and 92 per cent. of these were infected at the height of parasitaemia. This then is a Type D pattern.

Plasmodium fallax Schwetz, 1930

P. fallax of the Uganda tufted guinea-fowl was redescribed by Huff *et al.* (1950) and its pre-e generations demonstrated in pigeons. The cryptozoites and metacryptozoites resemble those of *P. relictum*. The metacryptozoites in the pigeon appeared to be degenerate and, despite the existence of an erythrocytic cycle, after heavy sporozoite inoculation no phanerozoites were seen. Huff, in a letter to Colonel Afridi, read to the Fifth Congress of Tropical Medicine and Malaria, has since announced that he has demonstrated phanerozoites.

Plasmodium circumflexum Kikuth, 1931

No pre-e development of this parasite has been demonstrated.

Although Hegner and Wolfson (1938b) failed to find phanerozoites in blood-induced infections in canaries, they have been described by Manwell and Goldstein (1939), Coulston and Manwell (1941) after single erythrocytic parasite infection, and Paraense (1952a). The type of development closely resembles that of blood-induced *P. gallinaceum* infections except that Manwell and Goldstein (1939) described elongate merozoites in the phanerozoites. This observation was not confirmed by Paraense (1952a) as is shown by his beautiful illustrations.

Plasmodium juxtanucleare Versiani and Gomez, 1941

No pre-e development of this parasite has been demonstrated.

Phanerozoic development of the *P. gallinaceum* type has been demonstrated in blood-induced infections of chicks by Barreto (1943) and Paraense (1947c). Natural infections in chickens showing *P. gallinaceum* type e-e schizogony has also been noted by Paraense (1947c).

Plasmodium durae Herman, 1941

No pre-e development of this parasite has been demonstrated.

Phanerozoites of the *P. gallinaceum* type have been noted by Purchase (1942) and Simpson (1944) in natural and blood-induced infections of turkeys.

Plasmodium hexamerium (=*P. oti*) Huff, 1935

No pre-e development of this parasite has been demonstrated.

Hegner and Wolfson (1938b) failed to find e-e forms in canaries, but phanerozoites of the *P. gallinaceum* type were demonstrated by Manwell (1951a) in blood-induced infections of the orange crowned warbler.

Plasmodium nucleophilum Manwell, 1935

No pre-e development of this parasite has been demonstrated.

Hegner and Wolfson (1938a, 1938b) reported phanerozoites of this species, but there seems to be some doubt if the infection used by these workers was a pure one. Manwell and Voter (1939), Goldstein (1939) and Manwell (1951a) have consistently failed to find e-e forms in blood-induced infections, probably owing to the lack of a suitable host for development of e-e schizogony.

Plasmodium elongatum Huff, 1930

No pre-e development of this parasite has been demonstrated.

The phanerozoic development of this parasite represents a considerable departure from the *P. gallinaceum* type so far described. This parasite and its associated plasmodia are usually accorded a place of their own in any considerations of the e-e cycle. To what extent such a differentiation is justified will be discussed later.

The e-e forms of *P. elongatum* were the first e-e forms to be described (Raffaele, 1934a, 1934b). The controversy which arose from various descriptions of their site of development has already been discussed. Phanerozoites have been demonstrated in sporozoite- and blood-induced infections by Raffaele (*1936a, 1937a, 1937b, 1938a, 1938b, 1939, 1940*), Huff and Bloom (*1935*), Porter and Huff (1940), Corradetti (1940e, 1940f, 1943), Corradetti and Gramiccia (1941a, 1941b, 1943), Wolfson (1940b), Tokura and Kawahata (1951), Garnham (1951a, 1951b), and personal observation (see fig. 4).

The cellular distribution of the phanerozoites represents the great difference between *P. elongatum* and *P. gallinaceum*. The cells parasitized by all forms of the parasite have been noted by Huff and Bloom (1935) and the percentage of parasites occupying them tabulated thus:

41%	in polychromatophil erythroblasts
29%	in basophil erythroblasts
20·5%	in haemocytoblasts
4%	in macrophages
2·5%	in normoblasts
1%	in monocytes
1%	in plasma cells
1%	in thrombocytes.

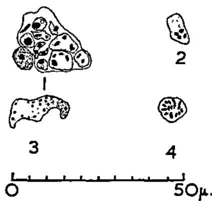


FIG. 4. Phanerozoites of *P. elongatum* in bone marrow smears. Stained with Giemsa. 1. Eleven immature e-e forms in an erythrocyte precursor. 2. 4-nucleate schizont. 3. Immature schizont. 4. Mature schizont having 15 elongate merozoites.

Scattered parasites are found in lymphocytes, erythrocytes, granulocytes and myelocytes.

The parasites produce pigment in erythrocytes, normoblasts and polychromatophil erythroblasts but not in basophil erythroblasts. It can be seen then that *P. elongatum* is essentially a parasite of unfixed cells and that the non-pigmented e-e cycle is largely in the erythrocyte precursors of the haemopoietic system, although 1 per cent. of the total parasites exist in fixed monocytes in the *P. gallinaceum* manner. The remainder are found in wandering macrophage cells in the *P. cathemerium* manner. The ratio of erythrocytic and other schizogony— $> 1 : < 99$, is not constant and erythrocytes may be heavily parasitized (Garnham, 1951a).

The production of pigment and the attack by schizontocides upon only those parasites producing pigment (Corradetti and Gramiccia, 1943) appear to offer the

only criteria for differentiating erythrocytic from e-e schizogony. Thus, typical erythrocytic schizogony occurs in erythrocytes, normoblasts and polychromatophils, whereas e-e schizogony occurs in basophils, haemocytoblasts and various white blood cells and monocytes. Precisely why the parasite should produce pigment in normoblasts and not in basophils seems obscure. Much or most of this development takes place in the bone marrow (see fig. 4).

The morphology of the e-e forms closely resembles that of the erythrocytic forms and they contain therefore at maturity some 4 to 30 elongate merozoites in a relatively small schizont (6μ). No micro- or macroschizogony has been noted.

The separate identity of *P. elongatum* e-e schizogony has been noted by many authors but doubt as to the real extent of difference from other types has been expressed by Mudrow-Reichenow (1949). Further to this, Garnham (1951a) has recognized two grades of development within the e-e phase of the species, one in more and the other in less primitive cells with all gradations between. Just because of these gradations, I incline to the view that not only can two grades not be distinguished within the species but that the existence of Type D *P. gallinaceum* e-e schizogony and *P. mexicanum* type e-e schizogony (to be described) denies to *P. elongatum* any real separate identity. For this reason any classification granting *P. elongatum* a separate generic identity is deprecated. This, however, ignores the description of *P. gallinaceum*-type e-e schizonts in the brain vascular endothelium by Tokura and Kawahata (1951) in Japanese wild birds infected with *P. elongatum*. This description would confirm the above postulations if it were accepted, but I feel it is more likely that the Japanese authors were examining an infection by two species. This statement is modified by the belief that one of the most important lacunae in the knowledge of the e-e schizogony is the lack of knowledge of the pre-e cycle of *P. elongatum*; a belief which is shared with Garnham (1948b) and Mudrow-Reichenow (1949).

Plasmodium vaughani Novy and MacNeal, 1904

No pre-e development of this parasite has been demonstrated.

Manwell (1947) reported e-e forms in a mixed infection of *P. relictum* and *P. vaughani* at a time when the blood contained predominantly *P. vaughani* forms, He felt at the time that these e-e forms were probably *P. vaughani*. Later, Manwell (1951a) reversed this opinion, presumably in the light of Sergent's observations of the persistence of e-e infections of *P. relictum*. Recently, Laird (1953) has described e-e forms of this parasite in a natural infection. The development is of the *P. elongatum* type displaying few merozoites and occurring mostly in basophil and polychromatophil erythroblasts. They occur to a lesser degree in normoblasts, macrophages and lymphocytes. This discovery destroys the groupings made by Manwell of the "small" avian plasmodia, *P. hexamerium*, *P. nucleophilum*, *P. vaughani* and *P. rouxi* as *P. hexamerium* displays a typical *P. gallinaceum*-type of e-e development.

Plasmodium rouxi Sergent, Sergent and Catanei, 1928

Neither pre-e nor e-e development of this parasite has been demonstrated, despite considerable study of it by Manwell (1951a).

The silence of the Sergent brothers on this subject would lead an observer to believe that e-e forms of this parasite cannot develop in blood-inoculated canaries as a strain of this parasite has been maintained by this means for years at the Pasteur Institute, Algiers. It is also worthy of note that *P. rouxi* blood-induced infections in canaries can be sterilized with ease by quinine and other schizontocides which indicates that no e-e development takes place (Curd, 1943; Manwell, 1951a). Such a development then awaits a suitable host and one might well hazard a guess that the development will be similar to that of *P. vaughani.*

Plasmodium huffi Muniz, Soares and Batista, 1950

No pre-e development of this parasite has been demonstrated.

Muniz *et al.* (1950, 1951) have described e-e schizogony in this species but the description of the type, though not the fact of development, is open to doubt. These authors described e-e forms of 6 to 30 merozoites dwelling in polychromatophils and other erythrocyte precursors in the bone marrow and in thrombocytes, lymphocytes and monocytes of the circulating blood. In addition, they described forms having up to 100 merozoites in the macrophage cells of the kidney, liver, spleen and lung. These observations led the authors to conclude that both *P. gallinaceum*-type and *P. elongatum*-type schizogonies were proceeding side by side in the manner of *P. mexicanum.* As far as could be judged it seemed that some 20 per cent. of the forms appeared in R-E cells and myelocyte series cells, while some 80 per cent. of the forms appeared in haemopoietic cells. If polychromatophils are accepted as hosts to the erythrocytic development, then their subtraction from the above figures resulted in an approximately 50–50 per cent. distribution in the above host cell groups. It must be admitted that, at the time, I entertained the idea that a double infection was being studied.

Very recently Huff (1953a) has studied preparations of this parasite sent to him by the Brazilian workers. He raised the issue of double invasion and reported Manwell as stating that the parasite in some of its forms bore a considerable resemblance to *P. nucleophilum.* Huff noted that many of the R-E host cells named by the Brazilian workers were not in fact parasitized and this led him to incline to the view that *P. huffi* is of the *P. elongatum* type rather than the *P. gallinaceum* or *P. mexicanum* type. He concluded that the parasite found in the bone marrow and spleen was indeed a new species, *P. huffi* rather than *P. nucleophilum.* This view was based to some degree upon the belief that *P. nucleophilum* will display a *P. gallinaceum* type of e-e schizogony by analogy with *P. hexamerium.* Unfortunately, Laird's work upon *P. vaughani* destroys the validity of this analogy by the dismemberment of the " small " avian group. Should *P. huffi* prove to be

a true species, then it apparently displays the *P. elongatum* type of e-e schizogony. Should it prove to be *P. nucleophilum* then the same applies to this species. No more can be said at this stage.

As no information is available concerning the e-e development of *P. polare* this brings to a conclusion the study of the morphology of e-e stages of avian plasmodia.

It is now necessary to study the e-e development of those saurian plasmodia about which information is forthcoming. The types of development, as far as they are known, appear to parallel types or combinations of types of avian plasmodia and will therefore be related to the patterns already described.

Plasmodium mexicanum Thompson and Huff, 1944

No pre-e development of this parasite has been demonstrated.

Phanerozoites have been observed in both naturally occurring and blood-induced infections of lizards by Thompson and Huff (1944), Thompson (1946), and Pelaez, Peres Reyes and Barrera (1948a, 1948b). The e-e schizonts are scanty in the natural hosts whether naturally or experimentally infected (Pelaez *et al.*, 1948b). In experimental hosts it was found that on blood inoculation both *P. gallinaceum* and *P. elongatum* types of e-e development occur (Thompson and Huff, 1944). All cells of the erythrocyte series may be infected with schizonts containing less than 30 merozoites when mature. Like *P. elongatum* only the pigment-producing parasites are attacked by schizontocidal drugs (Thompson, 1946). Phanerozoites are also found in granulocytes, myelocytes, lymphocytes, monocytes, thrombocytes and circulating macrophages.

At the same time phanerozoites, having up to 200 merozoites, can be found in R-E cells of the liver and spleen and in fixed macrophage and vascular endothelial cells (Thompson and Huff, 1944). The predominance of one pattern of development or the other changes from host to host. Thompson and Huff (1944) found that in *Sceloporus* sp. the predominant pattern of development was the *P. elongatum* type, whereas in *Crotaphytus* and *Phrynosoma* the pattern was the *P. gallinaceum* type. Thompson (1946) found abundant e-e forms of both types in blood-induced infections in *Sceloporus* while Pelaez *et al.* (1948a, 1948b) found only scanty e-e forms in natural infections of the same genus Palaez and his colleagues concluded that the parasite produced abundant e-e forms on experimental blood infection, especially in species other than *S. ferrariperezi ferrariperezi* and *S. microlepidotus microlepidotus* the natural host species. This probably indicates Type A and temporary Type C patterns of development.

The insect vector of the *Plasmodium* is unknown, and little more can be said of this pattern of development until the sporozoite-induced infections can be studied extensively in the laboratory. It might also be noted that nobody has ever questioned whether the above observations have been made upon a single

pure infection, as nobody has yet ventured to distinguish species on the basis of e-e development.

Plasmodium lacertiliae (synonym *P. lygosomae*) Thompson and Hart, 1946

No pre-e development of this parasite has been demonstrated.

I have been unable to ascertain sufficient points of difference between *P. lacertiliae* and *P. lygosomae* from the published works to warrant the differentiation of two species. As the natural hosts are closely allied the latter has been named here as a synonym of *P. lacertiliae* (Thompson and Hart, 1946).

Laird (1951) has reported e-e forms of this parasite in natural infections of skinks. The forms are found in thrombocytes and lymphocytes in the heart blood. Extracellular non-pigmented forms containing 20 chromatin pieces were also described by Laird. The pattern of this development is at present obscure, although Laird noted some similarities to *P. mexicanum*.

Plasmodium pitmani Hoare, 1932

No pre-e development of this parasite has been demonstrated.

Garnham (1950a) described phanerozoites of this parasite in *Mabuia*. They are found in fixed monocytes in the spleen and heart and contain, when mature, up to 100 merozoites. No e-e forms were found in erythrocyte precursors or other blood elements. Thus it seems that the development is of the *P. gallinaceum* type. The belief has already been expressed that two distinct grades do not exist in *P. elongatum* e-e infections and therefore I am disinclined to accept Garnham's (1951a) tentative proposal that *P. pitmani* may belong to the grade of *P. elongatum* pattern in which macrophage cells are affected.

Plasmodium agamae Wenyon, 1908

No pre-e development of this parasite has been demonstrated.

Garnham and Lyndhurst Duke (1953) have recently exhibited blood slides of this parasite showing e-e forms in monocytes of the peripheral blood. The schizonts would appear to produce not more than 30 or so merozoites. As yet nothing can be guessed as to this parasite's position in the still tangled skein of e-e schizogony patterns, but comparison with *P. lacertiliae* is interesting, if hazardous.

This brings to an end the account of our scanty knowledge of the e-e schizogony of the saurian plasmodia.

(*b*) CHEMOTHERAPY, BIOCHEMISTRY

Chemotherapy

As has been stated, chemotherapeutic considerations were one of the most powerful progenitors of the postulation of an e-e cycle in *Plasmodium*.

The Sergent brothers had reported that quinine, though clearing blood infections of *P. relictum*, exerted no prophylactic activity. Russell and Nono, and

Tate and Vincent, had reported similar effects with pamaquine in *P. cathemerium* and *P. relictum* infections.

With the demonstration of the e-e cycle in 1937 the efficacy of antimalarials against the e-e forms became visibly demonstrable and, as the e-e stages were known to be virulent, frequently causing death, so the survival periods and death rates of hosts also became criteria in the assessment of antimalarial activity against the e-e cycle.

By these means workers were able to demonstrate that quinine had no appreciable activity against the course of the e-e cycle, induced by sporozoites, trophozoites or phanerozoites, of *P. gallinaceum* (James and Tate, 1937a, 1938; Brumpt, Bovet and Brumpt, 1937; Ritis, 1940; Adler and Tchernomoretz, 1941, 1943; Missiroli, 1941b; Coggeshall *et al.*, 1944, Curd *et al.*, 1945; Paraense, 1946, 1947a, 1947b; Tullis, 1947; Lewert, 1948; Coulston and Huff, 1948; Greenberg *et al.*, 1950b; Baranger and Filer, 1951); of *P. relictum* (Missiroli, 1937b, 1941b; Curd *et al.*, 1945; Bishop, Birkett and Gilchrist, 1947); of *P. cathemerium* (Kikuth and Mudrow, 1939c; Mudrow, 1940; Scheng, 1943); of *P. elongatum* (Corradetti and Gramiccia, 1943); of *P. juxtanucleare* (Paraense, 1947c); of *P. mexicanum* (Thompson, 1946).

The same inefficacy was shown by mepacrine on the e-e cycle of *P. gallinaceum* (Brumpt *et al.*, 1937; Coggeshall *et al.*, 1944, Curd *et al.*, 1945; Tonkin, 1947; Coulston and Huff, 1948; and Greenberg *et al*, 1950b); of *P. relictum* (Archetti, 1941; Curd *et al.*, 1945; and Bishop *et al.*, 1947); of *P. cathemerium* (Kikuth and Mudrow, 1939; Mudrow, 1940; Scheng, 1943); of *P. elongatum* (Corradetti and Gramiccia, 1943) and by various other schizontocides in various infections: Certuna on *P. cathemerium* (Kikuth and Mudrow, 1939), Italachina on *P. relictum* (Archetti, 1941), a sulphone derivative on *P. gallinaceum* (Coggeshall *et al.*, 1944), chloroquine on *P. gallinaceum* (Greenberg *et al.*, 1950b), and pantothenic acid competitors on *P. gallinaceum* (Bracket, Waletsky and Baker, 1946).

A large number of drugs have been shown to exert some effect upon the e-e forms of avian plasmodia. These drugs vary considerably in efficacy and no one of them has been shown to be completely curative of sporozoite-induced attacks of all species of avian plasmodia if the sporozoite inoculum exceeds the contents of one mosquito's glands. (Very recent reports, however, indicate that pyrimethamine may be an exception to this.) The following chemotherapeutic agents have been demonstrated to have some appreciable effect upon the e-e cycle, either by direct microscopic observation or analysis of death rates and survival times of host animals. A useful summary of information up to 1948 is a paper of Coatney and Cooper (1948a).

A. *Poor activity*

1. *8-amino-quinolines*, including pamaquine, pentaquine, isopentaquine and primaquine. Activity at above toxic levels, however, is considerable (Kikuth and

Mudrow, 1939 ; Mudrow, 1940 ; Coggeshall *et al.*, 1944 ; Gingrich, 1946 ; Coggeshall and Porter, 1946 ; Bishop *et al.*, 1947 ; and Greenberg *et al.*, 1950b). Combination with quinine increases the efficiency of the 8-aminoquinolines (Greenberg *et al.*, 1950b). This indicates that the activity of this series upon e-e forms may in fact be quite high and death rate analysis is deceptive since the series is relatively inactive against the equally pathogenic blood cycle.

B. *Medium activity* (No true radical cure)

2. *Sulphonamides*, including sulphathiazole, sulphapyridine, sulphadiazine, sulphamerazine, sulphanilamide, sulphaguanidine, sulphapyrazine, sulphamethazine, sulphamezathine, sulphaquinoxaline, proseptasine, metachloridine and the metanilamide series (Coggeshall *et al.*, 1944 ; Americano Freire and Paraense, 1944 ; Coatney and Cooper, 1944 ; Thompson, 1945 ; Curd *et al.*, 1945 ; Brackett, Waletsky and Baker, 1945 ; Brackett, 1946 ; Brackett and Waletsky, 1946a, 1946b ; Paraense, 1946 ; Coatney and Sebrell, 1946 ; Coggeshall and Porter, 1946 ; Geiling and Taliaferro, 1946 ; Americano Freire, 1946 ; Davey, 1946 ; Gingrich, 1946 ; Hughes and Brackett, 1946 ; Maier, 1946b ; Gingrich, Schoch and Taylor, 1946 ; Zuckerman, 1946 ; Tonkin, 1947 ; Coulston and Huff, 1948 ; and Greenberg *et al.*, 1950b).

3. *Biguanides*, including proguanil and M4430 the paludrine precursor (Curd *et al.*, 1945 ; Davey, 1946b ; Coatney and Cooper 1948a ; Hawking, 1947 ; Hawking and Perry, 1948 ; and Greenberg *et al.*, 1950b).

4. A mixed group, including streptothricin and p-anisyl-guanide (Tonkin, 1946) and aureomycin (Coatney, Cooper, Greenberg and Trembley, 1949).

C. *Medium activity* (True causal prophylaxis in some species and not in others)

5. *Acridones*, including endochin (Stephen, Tonkin and Walker, 1945, 1947 ; Coatney and Sebrell, 1946 ; Gingrich, 1946 ; Coggeshall and Porter, 1946 ; Kikuth and Mudrow-Reichenow, 1947).

6. *Naphthaquinones*, including hydrolapichol. This series appears to owe its prophylactic activity to sterilization of sporozoites rather than e-e forms (Coatney and Sebrell, 1946 ; Gingrich, 1946 ; Coggeshall and Porter, 1946 ; Gingrich, Schoch, Schwab and Shepherd, 1947 ; Coulston and Huff, 1948 ; Clarke and Theiler, 1948).

7. *Triazines*, including the metabolic breakdown product of proguanil. Work is in progress upon these compounds but they are known to be active (Brackett and Waletsky, 1946 ; Carrington *et al.*, 1951).

D. *Apparent full activity* (True causal prophylaxis of light-sporozoite-dosage infections)

8. 2-4-*diaminopyrimidines*, including *pyrimethamine* (Falco, Goodwin, Hitchings, Rollo and Russell, 1951 ; Rollo, 1952 ; and Greenberg, Coatney and Trembley, 1953).

One further series might be mentioned which could be called the ethnographic group. Baranger and Filer (1953) have claimed to demonstrate partial prophylaxis in sporozoite-induced *P. gallinaceum* infections by means of collars of gold, copper and iron.

While perusing the wealth of published material on the chemotherapy of avian and saurian malaria a student might manage to extract some fruitful data pertaining to the e-e cycle. With reluctance, the question of schizontocides and pigment production is merely noted, as this phenomenon would appear to have in it the elements of a useful line of research.

No clear-cut indication is given as to the best mode of interference with the development of e-e forms. One manner of attack is obviously favoured. The inhibition of schizogony by interference with the enzyme system involving para-aminobenzoic acid (PABA), folic and folinic acids offers a certain route. The action of the sulphonamides, metanilamides, biguanides (triazines?) and the 2-4-diamino-pyrimidines all point this way. But we are left with the puzzle of the mode of action of the 8-aminoquinolines which seem to have some mysterious manner of entry into the parasite complex all of their own. The attack upon schizogony as such allowed by PABA, etc. inhibitors is not a mode much favoured by clinicians who have thereby to wait some 3 to 4 days before a clearance of blood parasites can be expected, although combination with an efficient schizontocide should eliminate this difficulty. Another objection should be added to the over-emphasis on attack of the PABA-folinic acid system. At present three lines of chemotherapeutic investigation are being vigorously pursued: (*a*) Triazines, (*b*) 2-4-diaminopyrimidines, (*c*) pteridines. Already cross-resistance has been demonstrated between (*a*) and (*b*) and it is possible that (*c*) too will prove to be of the same *genre*. There is at present a danger that over-emphasis upon a favoured mode of attack and lack of research along new lines will result in a series of drugs all effective against normal strains of malaria but all ineffective against the already numerous strains resistant to the first of the drug series. Such is the case now with the cross-resistance of *P falciparum* proguanil-fast strains to pyrimethamine. This leaves the world with only one effective drug for such strains—primaquine. One step ahead is insufficient lee-way in the fight between man and his parasites.

In all of the work detailed above there has been only one direct study upon the effect of drugs upon the pre-e stages of avian malaria. Coulston and Huff (1948) observed that the pre-e forms react to drugs much as do the phanerozoites. They noted some differences between cryptozoites and metacryptozoites, but as the former were confined to the wing skin while the latter were observed all over the body, particularly in organs richly supplied with drug-carrying plasma, such differences may not be surprising.

Two interesting questions which arise out of quinine therapy of blood-induced *P. gallinaceum* infections should be discussed. The first arises from the reports that quinine treatment increases the number of e-e forms and, to a lesser degree,

causes them to appear earlier in the infection (James and Tate, 1938, Paraense, 1946). Paraense noted that *in untreated birds surviving the* primary parasitaemia, death ensued after some 20–21 days, whereas it occurred in 12–15 days in quinine-treated birds having no erythrocytic infection.

The only possible explanation seems to be that if the parasite divides its forces between tissue and blood then it takes longer to kill its host. If forced by quinine to take refuge in tissue, the parasite multiplies rapidly there, unaffected by quinine, to the same parasitic level as it would have had it been unattacked. Such a level in one site or the other *is sufficient to kill the bird, but not if divided* between the two sites. The lag of say 5 days between death times for an erythrocytic site (7 days) and a histiocytic site (12 days) would be explicable in that 5 days are necessary for the parasite to "learn" to multiply exclusively in fixed tissue, i.e. to produce predominately histiocytic merozoites.

The other question must be taken in conjunction. It has been amply demonstrated that quinine, no matter when or how it is administered, is unable to prevent the growth of an e-e cycle in blood-induced infection of *P. gallinaceum*, *P. relictum* or *P. cathemerium*. This remains true, despite the fact that the inoculated *parasites are under immediate attack by quinine, in some experiments* already circulating in the new host. Thus, an e-e cycle is able to *establish* itself despite quinine attack upon its erythrocytic progenitors. There can be a variety of explanations of this problem.

(1) A natural resistance of some of the inoculated parasites has been postulated by Brumpt *et al.* (1937) and Bishop *et al.* (1947).
(2) An immediate invasion by circulating merozoites of fixed tissue has been suggested by Bishop *et al.* (1947).
(3) The concomitant inoculation of e-e parasites in wandering macrophages has been suggested also by Bishop *et al.* (1947).
(4) Some erythrocytic forms retire to the inner organs, there to resist quinine and invade tissue cells at their leisure.

(1) seems improbable, as such forms have not been noted *in the blood* 48 hours after inoculation. (3) in general has been discredited by the work with single erythrocytic parasite infections, although such infections have not been tested chemotherapeutically.

(4), or some variant of it, is obviously *favoured by Corradetti and his coworkers with their contention* that e-e forms appear more or less suddenly on or about the 11th day and their obvious preference for the Bignami theory of the genesis of relapse. On the *other hand, Tullis (1947)*has observed e-e forms on the 4th day after blood inoculation. Further, it should be remembered that only a few survivors with some inclination to tissue invasion *will initiate the tissue phase* immediately or later. If immediately, it will then take some time for such forms to grow and multiply and waste a proportion of their energies *in the production of*

haemotropic merozoites to be sacrificed to the quinine until it "learns" better and produces largely histiotropic merozoites. Such processes could well occupy some 7 or 8 days. The parasite adapted to histiotropic development would then become quickly visible on or about the 11th day. There is no reason to suppose that a careful search of the organs of a chick receiving a heavy blood inoculation and quinine treatment would not reveal e-e forms from the 4th day onwards.

If this problem is observed from another angle, the hypothesis of Corradetti and his colleagues demands that a very few retiring erythrocytic parasites should burst into sudden activity and produce very large numbers of e-e parasites in a short space of time without any prior "training" in such activity. I feel bound to accept the proposition (2) with its expansion as expressed above.

Biochemistry

In any consideration of the biochemistry and physiology of the e-e forms of avian malaria there is no want of apparently promising leads to follow, which no doubt contain in their number a high proportion of blind alleys. A student in this field might well feel dazzled by the number of loose ends and odd pieces of information demanding synthesis into some rational system. Unfortunately it is often the case that such unfinished work and untrimmed data are the result of a fundamental lack of basic biochemical knowledge : knowledge which will be more easily obtained from more manageable cell systems. In general it has proved less difficult to obtain biochemical principles from organisms other than *Plasmodium*, especially systems grown *in vitro*, and subsequently to apply the general principle to the study of *Plasmodium*. When a fact is discovered about *Plasmodium* which is ahead of general biochemical knowledge, it is therefore left suspended, as it were, until biochemical principles catch up with it and finally absorb it. Thus, ventures into this particular terrain require firm ground underfoot for a wary investigator.

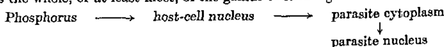
Some definite facts and a few attractive hypotheses are available, culled from chemoprophylaxis and its inhibition, from histochemical observation and from various dietary experiments. As has been shown above, drugs of one group active against e-e forms display an essentially similar mode of action. It is found that sulphonamides, proguanil and pyrimethamine all act on the parasite through inhibition of the later stages of schizogony and nuclear division (Black, 1946a, 1946b, 1949 ; Hawking and Perry, 1948 ; Thurston, 1951 ; MacFadzean, 1951 ; Goodwin, 1952 ; Schmidt and Genther, 1953). It is known that strains of *Plasmodium* spp. resistant to sulphadiazine are insensitive to proguanil (Bishop and McConnachie, 1948), and those resistant to proguanil are to some extent, or wholly, insensitive to pyrimethamine and *vice versa* (Schmidt and Genther, 1953 ; Thurston, 1953). Moreover, it is known that the activity of sulphonamides is reversed by equimolar or lesser amounts of para-aminobenzoic acid, that of proguanil by folic acid and purines, and that of pyrimethamine by folic and folinic

acids and by liver extract (presumably purines and vitamin B_{12}) (Marshall *et al.*, 1942; Falco *et al.*, 1949; Hitchings, 1952).

From the chemical and biochemical side of the problem it can be shown that there are certain distinct structural relations between the drugs and between the antagonists and between the two groups. Further, it is known in a general sense that the enzyme complex catalysing the production of purines, nucleosides and eventually nucleic acids and the exchanges between and within these groups can be summarized thus: p-aminobenzoic acid→pteroylglutamic acid (folic acid)→ folinic acid; this last substance is then involved in the catalysis of reactions forming thymine and probably other purines (Lampen and Jones, 1946; Hitchings, 1952; Jacobsen, 1952). This system is obviously vital to the parasite and may be interfered with at various levels by the above drugs. The actual interference appears to be concerned with the exchange relations existing between ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) which come into play at the time of nuclear division which requires a transfer of nucleic acid to the chromatin in the form of DNA in increasing amounts.

This complex is undoubtedly at work in the e-e parasite and the extent to which the parasite may be affected or attacked by experimental procedures while in this state will be largely determined by pharmacodynamic and physiological considerations involving both qualities of the agent and the host tissue. Thus, the inefficacy of sulphadiazine in certain e-e infections is more likely to be bound up with availability of drug in the parasitized area and the biochemical peculiarities of the host tissue than with any modification of the above enzymatic complex in the e-e stages.

It is at this point where conjecture can be given greater foundation by means of direct histochemical and biochemical observation on the parasites themselves. Lewert (1952a) has shown that the DNA content of the e-e parasite chromatin reaches by gradual stages its full amount at the merozoite stage as measured by the intensity of Feulgen staining and toluidine-blue staining of schizonts treated with ribonuclease. Further, Lewert (1952b) has shown that host-cell nuclei rapidly lose RNA to the parasite and he assumed that this RNA was absorbed into the parasite cytoplasm. However, Clarke (1952) has shown that labelled phosphorus taken up by normal cells as RNA is taken up by parasitized cells as parasite DNA. Further, this DNA uptake can be directly correlated with parasitic growth, presumably with progressive nuclear division. Clarke assumed that the uptake of labelled DNA was direct. On the other hand, from the evidence available, it would seem more reasonable to assume that phosphorus taken up runs the whole, or at least most, of the gamut of exchange thus:



the last step involving production of generative DNA.

Lewert (1952a) has shown that active division in the e-e schizont of *P. gallinaceum* is accompanied by some alkaline phosphatase activity, as would be expected. He also demonstrated by alcoholic differentiation of schizonts stained with toluidine blue that metachromatic granules appear in the cytoplasm in the position of aggregation of basophilic substance probably RNA and removed by alcoholic differentiation. He found that the number of granules increased in time with nuclear divisions, which indicated a cyclical change in RNA cytoplasmic concentration equivalent to that of nuclear DNA. The foci of alkaline phosphatase activity were associated with these metachromatic granules.

Both Lewert (1952a) and Chen (1944) have described Feulgen-stained DNA formations in the nucleus which might be interpreted as chromosome development. My personal observations have been in general accordance with those of Lewert except in two particulars. Firstly it is not believed that toluidine-blue staining of nucleus represents staining of DNA exlusively after eradication of RNA with ribonuclease. Nuclei of immature schizonts stain well by this method when the Feulgen reaction is almost negative. Toluidine blue is obviously staining constituents of the nucleus unaffected by ribonuclease other than just DNA. Secondly, metachromatic granules have not been demonstrated in toluidine-blue-stained alcohol-differentiated sections of liver infected with e-e forms of *P. gallinaceum*. It is probable, however, that the less compact forms seen in brain smears and used by Lewert may be more suitable to this technique.

Apart from this particular nucleoprotein system with its associated enzyme complexes (e g. vitamin B_{12}) very little biochemical information is available relating to e-e forms. Brackett *et al.* (1946) have shown that pantothenic acid is unnecessary for e-e parasitic growth, although essential to erythrocytic forms. Various observations lead one to believe that pyridoxine, biotin, vitamin C and nicotinic acid are not essential to the e-e forms, but it is not always wise to infer conclusions from experiments designed to observe the effect of dietary factors on erythrocytic forms. It would be useful to know precisely why the proguanil-fast strain of *P. relictum* produced more e-e forms than did the parent strain (Redmond and Fincher, 1949).

(c) RELAPSES, IMMUNOLOGY, PATHOGENICITY

Relapses:

That a persisting e-e cycle is the true cause of relapse in malaria has been postulated many times, and that it represents, as long as it persists, a potential source of erythrocytic parasites in avian malaria cannot be doubted.

Most of the work done on this and the related subject of immunology has been carried out with *P. gallinaceum* infections often blood-induced and in chicks, which cannot be accepted as providing results always applicable in a general sense. Firstly, *P. gallinaceum* is a highly virulent infection in the young chick, killing on

blood inoculation as a rule, thus the consideration of relapse, a phenomenon best studied in milder infections of less susceptible hosts, is complicated by heavy death rates and a highly active erythrocytic parasite. Secondly, blood-induced infections of this parasite do not follow a normal course of development and the e-e development is distorted.

These qualifications should be borne in mind in the ensuing discussion.

Many authors have postulated that the e-e forms are the cause of relapses both in the particular avian sense and more generally, at a time when only the avian e-e forms had been demonstrated (James, Kikuth and Mudrow, etc.). This tendency to argue from the particular to the general, usually involving the consideration of relapsing *P. vivax* malaria of man, has resulted in a somewhat confused picture, leaving a general conclusion in a student's mind that relapse is a commonly observed phenomenon in avian malaria. This cannot be accepted as generally true and the relapse phenomenon in avian malaria in the strict *P. vivax* sense, involving a negative blood phase, is probably not such a common laboratory phenomenon as one would be led to believe. A relapse is taken to be a reappearance of blood parasites after an apparently negative blood phase, such a reappearance taking place after an appreciable time since the former parasitaemia. Any more closely argued definition of a relapse in avian malaria will lead into ill-reconnoitred fields and it is unfortunate that such a loose term as "apparently negative" must be adopted to allow further discussion. During this apparently negative blood phase the possibility of a low-level erythrocytic schizogony is not ruled out. The problem of genesis of relapse therefore becomes a question of whether the reappearance of blood parasites is initiated largely by merozoites from the tissue phase or largely by those of the blood phase. In this work the phenomenon will be termed a relapse if largely initiated by e-e merozoites despite the presence of erythrocytic schizogony concomitantly. It will be termed a recrudescence if largely initiated by erythrocytic merozoites. I am painfully aware that this adds yet another definition of what constitutes a relapse, but it seems the only convenient theoretical stand that can be taken in the avian malarias, according to the present store of knowledge. It should be stressed, however, that this definition is theoretical and biological and confined to the avian malarias. It is not intended to replace other convenient definitions in other spheres of malariology such as the clinical field, or indeed to cover human malarias, which will be dealt with elsewhere.

In the light of this theoretical standpoint, the "relapse" phenomenon of *P. gallinaceum* can be discussed. That such a phenomenon occurs in the laboratory infection of the fowl is well known to any worker who has had to deal with this parasite. Investigation has been directed inevitably at one of two theories of causation. The one, the theory which holds that relapses are caused by an underlying e-e schizogony, has always been held with one eye, as it were, upon human malaria, and has therefore never been adequately studied in avian malaria. The

other is the original postulation of Bignami (1913) that the phenomenon is the result of a reactivation of a persistent low-level erythrocytic schizogony. The main advocate of this theory has been Corradetti, who has built up a theory of the genesis of e-e forms in blood-induced *P. gallinaceum* infections designed largely to show that e-e forms are a transient and even accidental phenomenon and, as such, are not the cause of " relapses ".

The contentions of Corradetti may be summarized thus (Corradetti, 1938c, 1940c, 1940e, 1940f, 1940g, 1940j, 19141a, 1941b, 1942a, 1942c, 1943, 1949, 1952; Corradetti and Cavaceppi, 1952): in blood-induced or phanerozoite-induced *P. gallinaceum* infections the e-e forms do not appear until 8–11 days after infection. On appearance they exist only until the 25th–30th day after infection. During these first 11 days in blood-induced infections the R-E system engulfs pigment but not whole parasites or parasitized erythrocytes. At about the 11th day the R-E system becomes activated to engulf parasites and whole parasitized erythrocytes. An equilibrium between parasite and R-E system is then set up in which the R-E system loses some of its " parasitolytic activity " and reaches an accommodation with the parasite which allows the appearance of the e-e cycle. Such a loss of lytic powers of phagocytes was first postulated by Danilewsky (1890), Bignami (1891) and Golgi (1893). This state of equilibrium is only transitory. When immune responses catch up with the rate of parasite development " parasitolytic activity " begins to eradicate the tissue phase parasites. This eradication is complete by 25–30 days after infection even by sporozoites. Erythrocytic parasites may linger after this time but at no time after this will e-e forms be seen in the absence of erythrocytic forms. The tissue phase of the infection is not seen again as the immune response to them, now stimulated, persists and it is impossible to superinfect tissue by phanerozoite inoculation (Cavaceppi, 1951).

Other Italian workers (Oddo and Bruno-Smiraglia, 1951; Alessandro, Oddo and Smiraglia, 1948) have shown that pigment persists in certain organs during latency of *P. gallinaceum* infections, and therefore consider that latent erythrocytic forms exist and could account for all clinical phenomena at this stage, including " relapses ".

The bearing which the sequence of events postulated above has upon relapse is obvious. If a " relapse " occurs under the conditions mentioned, it is due not to any persistence of an e-e cycle and therefore must be due to a persistent erythrocytic cycle. Thus the phenomenon becomes a recrudescence and not a relapse.

Corradetti's theory must not go by without challenge. No less than six objections might be listed.

Firstly, it is a compound of observations carried out with every type and mode of inoculation, of which by far the most often used are the blood and phanerozoite inocula. Not only are these methods unnatural, but also the chick host used cannot be accepted as an ideal host. It must be added that it appears, from much

of Corradetti's work, that fowls must have been used to some extent. The present author holds it unwise to assume that phenomena *observed in such infections will* occur in a similar manner in natural infections, and this essentially is Corradetti's thesis. This is emphasized by the definite knowledge that much of the foregoing is incorrect for sporozoite infections where e-e forms are visible continuously.

Secondly, e-e parasites *do* appear in the R-E system prior to 8 days, particularly if the blood inoculum is heavy and quinine is used to suppress parasitaemia (Tullis, 1947 ; Refaat, 1953).

Thirdly, if it is assumed that e-e forms do not appear for about 11 days and then in large numbers, what are the parasites doing during those days in quinine-treated infections when no erythrocytic parasites are visible in the peripheral system? A low-grade erythrocytic schizogony continuously suppressed by quinine could hardly account for the flooding effect on the 11th day.

Fourthly, too much store should not be set upon the observation of the persistence of malarial pigment. This substance is notoriously persistent at any time and may be seen up to a year after radical cure of malarial infections. That recently laid-down pigment can be distinguished seems to be a dubious point.

Fifthly, it should not be forgotten that much of Corradetti's observation has been on brain material where the parasites lie in vascular endothelium. As has already been stated, the change-over from R-E cells to vascular endothelium occurs late in the infection and thus prolific parasitic development in the brain may be expected late in the e-e development of the parasite.

Sixthly, there is a point which is puzzling and to which some answer is desirable. That an endothelial cell may easily engulf pigment and not whole parasitized red cells containing trophozoites or schizonts is readily understandable. On the other hand, presumably only a merozoite or a mature schizont may initiate an e-e infection on engulfment. Under such conditions it is not thought that a pigment-engulfing and possibly residual-body-engulfing phagocyte would refuse a merozoite. Whether or not it phagocytoses whole parasite-containing red cells is thought to be irrelevant, as it is assumed that an engulfed trophozoite already metabolizing haemoglobin would die in the new haemoglobin-free environment.

None the less, this theory of the genesis and significance of e-e forms has received the support of Chortis (1938a, 1938b, 1938c,) Taddia and Viero (1940), Knoche (1941), Verolini (1949), Ascoli and Alessandro (1950, 1951) and Grignaschi (1952).

In quinine-treated blood-induced infections of *P. gallinaceum* an alternative theory might be that e-e infection is immediately established but produces largely haemotropic merozoites which are killed by the quinine. (In the case of untreated birds, immune responses at the stage of declining parasitaemia would take the place, to a lesser degree, of quinine.) After some delay, this state of affairs, unsatisfactory to the parasite, forces upon it a histiocytic merozoite production, with the consequent 11th day flooding effect. Such a changeover in development would

take place after 4, 5 or 6 generations of futile, largely haemotropic merozoite production, being the original character of the inoculated parasites. After this period the parasite "learns its lesson" in survival and produces largely histiocytic merozoites. Such a theory would go some way towards explaining why a quinine-treated bird may die from an e-e infection on blood inoculation but survive both erythrocytic and subsequent patent e-e infection when untreated. The effect of earlier production of e-e forms in quinine-treated birds is also explaind by the more rigorous "lesson" being administered. Such an explanation suffers besides Corradetti's theory in being less precise and largely theoretical and it must be said that an addition can be made to Corradetti's postulation which seems to improve it, though it is doubtful if it would be accepted by that author.

Meyer (1949a) has shown that sub-patent e-e infections of the circulating blood definitely occur and, as such, are infective. Thus it may be that e-e forms seen before 8 days are the result of infection by inoculated sub-patent e-e forms in wandering macrophages and the 11th day flooding effect is none the less due to an activation of the R-E system as postulated by Corradetti. This goes some way towards removing a number of the objections to the postulation, but other objections still exist.

In summary it can be said, rather despairingly, that in *P. gallinaceum* infections as they are at present examined the twin phenomena of relapse and recrudescence are so intimately bound up that differentiation is impossible. It will be necessary to make a systematic study of the relapse phenomenon in Type A *P. gallinaceum* infections in partially immune hosts before this problem can be clarified.

The situation with regard to *P. relictum* is rather clearer although this may be due to the relative paucity of information available. It is known that long-term and apparently true relapses occur in the Italian strain (Draper, 1953) and the matutinal strain of *P. relictum* (personal observation) and that e-e forms have been described in the Algerian strain 2½ years after infection and a period of no parasitaemia (Sergent, 1949b). It seems therefore that *P. relictum* displays a true relapse phenomenon initiated by e-e forms (Reichenow and Mudrow, 1944; Sergent, 1949a, 1949b). Sergent (1949b) takes this thesis further into a general conclusion concerning the whole of the Plasmodiidae. He assumed a continuous histiocytic development of e-e forms until death of the host, or eradication of the parasite, thus ensuring the conservation of the strain in the host and the production of relapses designed to perpetuate the species.

The type of e-e development displayed by *P. elongatum* automatically places any renewed parasitaemia which may occur in the recrudescence class, owing to the close cellular relation existing between the two sites of parasite development. Indeed infections are more prone to chronicity than any real interruption of erythrocytic development, although some infections may become almost exclusively confined to cells of the haemopoietic system.

Immunity

Acquired Immunity

There is some disagreement about the degree of immunity excited by avian e-e forms. Paraense (1946, 1947, 1952b) holds the view that the e-e forms of *P. gallinaceum* do not excite any immune responses, and that antibodies produced by an infection relate to and affect only the blood phase. On the other hand, Corradetti (1942a, 1943, 1949, 1952) has shown that phanerozoites or sporozoites are unable to set up an e-e infection in birds in the latent phase of *P. gallinaceum* infections after the eradication of both e-e and erythrocytic forms by immune reactions. Corradetti (1949, 1952) and Corradetti and Cavaceppi (1952) have also shown that it is impossible to superinfect tissue of birds recovered from a Type B infection while erythrocytic forms appear as normal, and that splenectony has no effect upon such an immunity.

On theoretical grounds it would be logical to assume a considerable development of immune response. Intense e-e parasitization activates a considerable proliferation of the R-E system and such an activation and " parasitolytic activity " might well be expected to last for some time. Furthermore, should the e-e development continue at a low level, premunition would account for a continued immunity. Such a resistance to subsequent heavy endothelial superinfection is displayed by *P. relictum* (Draper, 1953). It would also be logical to assume that the immune responses to e-e forms are different from the immune responses to erythrocytic forms, and that immunity to the erythrocytic forms may be inactive against tissue forms of *P. gallinaceum*.

Such, however, may not be the case in *P. elongatum* where such immune responses as exist affect both phases, though to different degrees, depending upon the site of development and availability to immunity action. The immunologically sluggish bone marrow, " the stagnant backwater " as Garnham has called it, protects to some degree the e-e forms of *P. elongatum* and chronic bone marrow infections are common. This would be emphasized by depression of leucopoietic activity resulting from hyperplasia of the haemopoietic system. The special position of *P. elongatum* in this regard has been noted by Corradetti (1949).

Somewhat surprisingly the immunity set up by phanerozoites of *P. gallinaceum* does not affect the pre-e forms. Huff and Coulston (1946) were able to observe normal developing pre-e forms of *P. gallinaceum* in wing skin of chickens and ducks previously infected by repeated inoculation of sporozoites and trophozoites. This is inexplicable if one accepts that metacryptozoites are to all intents and purposes similar to phanerozoites, particularly with regard to sites of development. Russell, Mulligan and Mohan (1941) have shown that inactivated sporozoite inoculations conferred some agglutinogenic protection against sporozoite inoculation in *P. gallinaceum* infections. It seems therefore that the sporozoites inoculated by Huff and Coulston should have encountered some specific sporozoite agglutination factor and that the surviving cryptozoic merozoites should have encountered an

endothelial immunity conferred by previous metacryptozoites and phanerozoites. Several assumptions must be made if Corradetti's postulation of specific immunity to e-e development is to be retained, which indeed is intended. It is certain that the enormous numbers of sporozoites routinely used by Huff and Coulston would survive in reduced numbers the weak specific sporozoite agglutinin and also to an unknown degree any residual immunity to cryptozoic merozoites. It could be argued that by accident Corradetti's experiments involved a premunition due to existent e-e forms whereas Huff and Coulston's work did not, but such an argument is not felt to be necessary and, in view of Corradetti's careful search for such forms, the argument is discounted.

The point most worthy of note is that the observation of metacryptozoites by Huff and Coulston was made on wing skin and not in other organs. In wing skin antibody supply may be low but, as Huff and Coulston have pointed out, Taliaferro and Bloom (1945) failed to infect immune monkeys with intradermal inoculations of *P. knowlesi* blood forms. More important is the objection that immune response to e-e forms is likely in such tissue to be to some degree local and, as immunity response was never tested by repeated sporozoite inoculation into the same site, it seems that humoral and cellular immunity would be at a minimum in this particular area unless previous inflammatory reaction was provoked. Eventually the *P. knowlesi* blood forms must infect the whole blood system to " take " but in the case of pre-e forms of *P. gallinaceum* only the local tissue is affected.

Much of the above theoretical discussion is based upon the acceptance of Corradetti's " parasitolytic activity " as an immune response to an established e-e infection. Another view might be taken that in fact there is no such activity and all immune reactions are directed against the extracellular circulating merozoite stage of the e-e cycle. No definite information is available on this point.

Natural Immunity

There are, no doubt, many cases of natural immunity to phanerozoite infection, if all reported accounts of failures to infect various hosts with avian and saurian e-e forms are taken into account. Included among these are probably *P. rouxi* and *P. floridense* which it is felt await only a suitable host for the demonstration of their e-e forms (*vide P. hexamerium* and *P. vaughani*).

Natural immunity to pre-e forms has been studied by Huff and his co-workers (Huff and Coulston, 1946 ; Huff, Coulston, Laird and Porter, 1947 ; Huff, 1948d ; Coulston and Huff, 1948 ; Huff, 1951.) They have found a natural immunity to sporozoite infections which can be tabulated as shown in table 1.

It will be seen from this tabulation that natural immunity seems to bear no relation to host classification. For instance, *P. gallinaceum* thrives in the chicken but not in the guinea-fowl, another gallinaceous bird, and yet lives in the turkey and even in the canary. *P. relictum* shows all grades between full infectivity and non-infectivity in pigeons of various species within the same genus.

TABLE 1

– complete immunity + partial immunity
+ + slight immunity + + + no immunity

Host	Parasite							
	P. gallinaceum		*P. relictum*		*P. cathemerium*		*P. lophurae*	
	Tissue	Blood	Tissue	Blood	Tissue	Blood	Tissue	Blood
Chicken	+ + +	+ + +	–	–			+ +	+
Guinea fowl	–	–	–	–			+ +	+ +
Turkey	+ + +	+	+ + +	–			+ +	+ + +
Pheasant	+ + +	+	+ + +	–			+ + +	+ + +
Quail	–	–						
Duck	+ +	+	+ +	–	+ + +	–	+	+
Goose	+ + +	– or +						
Canary	+ + +	–	+ + +	+ + +	+ + +	+ + +	+ + +	+
Pigeons of Various Species	–	+	+ + + ↓ –	+ + ↓ –			–	+
Doves of Various Species	–	–	+ + + ↓ –	+ + ↓ –	–	–		
Pigeon-Dove hybrid			–	–				

MOST RESULTS REFER TO YOUNG BIRDS.

Huff and Coulston (1946) and Coulston and Huff (1948) have postulated a tissue-blood barrier to explain those cases where pre-e development is normal and no parasitaemia or only a low-grade parasitaemia has ensued. Whether such a barrier is similar to that which prevents the establishment of parasitaemia in many of the same hosts on blood inoculation is doubtful, as, in the case of *P. relictum*, Huff (1948d) has shown that it is possible to induce by blood inoculation but not by sporozoite inoculation a parasitaemia in doves. This presents an interesting analogy to *P. berghei*, where blood infection of mice is notoriously easy

whereas sporozoite infection is difficult (Vincke and Lips, 1950) though not impossible (Vincke and Peeters, 1953).

Some aspects of these general immunological problems have recently been summarized by Nauck (1953).

Pathogenicity

There can be no doubt that the e-e forms of avian and saurian malaria are virulent to their respective hosts as far as we know them, and if allowed to proliferate they may cause death. An uneasy accommodation no doubt can be achieved between host and e-e parasite in chronic infections, particularly in *P. elongatum* infections. The pathogenicity of these forms is amply illustrated by the Type B infections of *P. gallinaceum* where the host dies of an overwhelming e-e development without the intervention of the erythrocytic stages. Histologically, in such cases, the e-e parasites can be seen to be completely blocking the brain, causing a consequent fatal cerebral anoxia with all the usual symptoms. No doubt blockage elsewhere, particularly in the lung capillaries, causes a general anoxaemia and tissue anoxia which will reinforce the decisive cerebral anoxia.

Paraense (1946, 1947a, 1947b) has shown experimentally that the e-e cycle of *P. gallinaceum* in quinine-treated blood-induced and sporozoite-induced infections in chicks is the cause of death by the 10th to 17th day. In cases of untreated sporozoite-induced infections of *P. gallinaceum* in young chicks when one mosquito-equivalent inoculum is used the e-e cycle is the direct cause of death though aided indirectly by the blood phase (Coatney, Cooper and Trembley, 1945b). In this type of infection death follows only 3–4 days after the appearance of parasitaemia at a time when e-e forms are numerous in the cerebral capillaries.

(*d*) TISSUE CULTURE

Two excellent summaries by Porter (1948) and Hawking (1951) exist upon the subject of tissue culture of avian e-e forms. Another shorter review has been published by Meyer (1949b)

The first attempts to grow e-e forms in tissue culture were made by Huff and Bloom (1935). They attempted to culture bone marrow infected with *P. elongatum* in plasma. They found that the culture including plasmodia survived for 48 hours without development. The first major attempt to cultivate in tissue the *P. gallinaceum* type of e-e phase was that of Gavrilov *et al.* (1938) who used methods previously successful in *Leishmania* studies. These workers made various essays involving adult spleen, mesentery and muscle and embryonic bone marrow which they attempted to infect by bathing the explants in blood parasitized by *P. gallinaceum* They also tried to grow infected bone marrow *in vitro*. All these attempts were histologically negative, but the infected bone marrow was infective 10 days after explanation. The methods involved slide culturing with plasma-embryo-extract clots.

Hawking (1951) believes that the comparative lack of success in this series of experiments was due to the use of slide cultures, but at least two further imponderables are involved and may be of some importance. Firstly, if bone marrow was explanted early in the donor's infection it is possible that e-e forms were not present, or were in insufficient numbers. Secondly, the success on infection at the 10th day may have been due to persisting erythrocytic forms which survived longer than normal in a medium containing actively growing haemopoietic tissue. Thus it may be worth while, when considering growth of erythrocytic forms *in vitro*, to test the efficacy of a two-cell system containing growing bone marrow in conjunction with erythrocytes.

Hegner and Wolfson (1939) were partially successful in producing demonstrable parasites of *P. cathemerium* in tissue culture. They used hanging drop and roller-tube cultures of infected brain, lung and spleen. Although no parasites were demonstrated in explants at 8 days, infection of birds was successful. On the 15th day, however, very scanty e-e forms were seen in an explant of lung macrophages cultivated by the hanging drop method.

Rodhain *et al.* (1940) and Paraense *et al.* (1942) tried to infect embryonic tissue *in vitro* with sporozoites of *P. gallinaceum* but all results were uniformly negative. Coulston (1940) devised a novel method of cultivating erythrocytic forms by means of a semi-permeable sac in the abdominal cavity of the bird. During studies on these cultured forms one e-e parasite was seen.

The first uniformly successful results were those of Hawking (1944, 1945, 1946), Tonkin (1946) and Hawking and Tonkin (1947). These workers successfully cultivated e-e forms of *P. gallinaceum*, *P. relictum* and *P. lophurae in vitro*. The growth in all cases was luxuriant and was displayed to the best advantage in infected spleen explants. Brain, liver, bone marrow and buffy coat were also used with varying success. The techniques involved the use of Carrel-flask cultures with cover-slip bases for the explant. Cover slips were cemented with plasma and the explant was fixed to the slip with homologous plasma and chick-embryo-extract clots. The fluid phase consisted of 80 per cent. Tyrode solution and 20 per cent. homologous serum with small amounts of phenol red and penicillin. The optimum concentration for penicillin was thought to be 5u./ml. The addition of 1 to 5 per cent. embryo extract was found to assist growth but larger amounts preferentially encouraged unwanted fibroblasts. The fluid medium was changed every 5 days. The *p*H was maintained at 7·6 to 7·8 and adjusted by carbon dioxide. Successful cultures of *P. gallinaceum* were made from spleen taken one hour after sporozoite inoculation of the donor bird. Infective cultures of *P. gallinaceum* were maintained up to 89 days.

The e-e forms themselves appeared much the same as *in vivo*, though perhaps a little more vacuolated. They were found in macrophages rather than in undifferentiated fibroblasts. Hawking noted both dimorphic merozoites and cytomere formation in cultures of *P. gallinaceum* but both these observations should be

treated with reserve. No sausage-shaped forms such as are seen in brain vascular endothelium were seen, which indicates that the nature of the host cell controls the dimensions of the parasite to a considerable degree. Hawking also reported the presence of prominent inclusion bodies in association with the nuclei and generally of the same number. Lewert (1952) has also noted such granules *in vivo* and shown them probably to be aggregations of RNA. It has been noted that these granules are more prominent in tissue culture than *in vivo* (personal observation).

Zuckerman (1946) found that infected embryonic tissues gave better results than adult tissues. She used bottomless Carrel flasks and large cover slips cemented to the flasks, the technique later to be used with conspicuous success by Dubin and his colleagues. Porter (1948) reported success also with this and other methods, but recorded his failure to infect clean explants with either sporozoites or blood forms.

Meyer (1947), in a note, gave a rather equivocal description of infection of clean embryonic brain explants with blood forms of *P. gallinaceum*. However, she has since decided that these e-e forms were initiated not by erythrocytic forms but by e-e forms present in the wandering monocytes of the blood (Meyer, 1949a, 1953, Xavier de Oliveira, 1950). These two workers have shown that it is impossible to infect cultures with infected blood until late in the blood infection when e-e forms can be demonstrated after long search in the monocytes.

Lewert (1950a) has shown that the fluid medium as well as the tissue itself is continuously infective in cultures of e-e forms of *P. gallinaceum*. It is assumed that this is due to circulating merozoites. Lewert was unsuccessful in attempts to infect blood *in vitro* with such merozoites. Thus there seems to be some definite biological differentiation between the blood-invasive and tissue-invasive forms, which provides a barrier to change of tropism except under certain conditions prevailing *in vivo*.

Success with *in vitro* growth of e-e forms of *P. gallinaceum* in previously infected tissues has been reported for brain up to 134 days by Meyer and de Oliveira (1947), for heart, choroid plexus and pia mater up to 105 days by Lewert (1948b and 1950a) in both Carrel flasks and roller tubes. Gramiccia and Black (1948) have cultivated e-e forms from spleen from blood-infected birds. Dubin (1947, 1950, 1952) and Dubin and Yen (1950) have investigated the various factors influencing growth of e-e forms in spleen and buffy coat explants. They found the following conditions to be optimum :

Osmotic pressure	7–11 atmospheres
Fluid Phase	50% serum, pH 7·6
Antibiotics	100 u./ml. penicillin
	100 μgm./ml. streptomycin

Xavier de Oliveira (1950) was able to maintain e-e forms of *P. gallinaceum* in blood macrophages from an infected bird for 5 months. He was also able to show

Hawking (1951) believes that the comparative lack of success in this series of experiments was due to the use of slide cultures, but at least two further imponderables are involved and may be of some importance. Firstly, if bone marrow was explanted early in the donor's infection it is possible that e-e forms were not present, or were in insufficient numbers. Secondly, the success on infection at the 10th day may have been due to persisting erythrocytic forms which survived longer than normal in a medium containing actively growing haemopoietic tissue. Thus it may be worth while, when considering growth of erythrocytic forms *in vitro*, to test the efficacy of a two-cell system containing growing bone marrow in conjunction with erythrocytes.

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Antibiotics	100 u./ml. penicillin
	100 μgm./ml. streptomycin

Xavier de Oliveira (1950) was able to maintain e-e forms of *P. gallinaceum* in blood macrophages from an infected bird for 5 months. He was also able to show

that e-e forms are definitely present in the circulating blood late in the blood infection and that these forms, not the erythrocytic forms, were initiating the cultivated e-e infection. Meyer (1953) has reported cultures infective up to one year.

Numerous authors up to 1949 had reported failure to infect cultures with sporozoites. This was achieved by Dubin, Laird and Drinnon (1949, 1950), using sporozoites of *P. gallinaceum*. They grew clean embryo spleen explants for 4 days before sporozoite inoculation, thus allowing full macrophagic activity to develop in growing macrophage cells. On inoculation with sporozoites they were able to demonstrate cryptozoites and metacryptozoites *in vitro* after 48 and 72 hours. For this work Carrel flasks were used after the methods of Zuckerman (1946). Antibiotics were used to ensure sterility and the sporozoites were obtained without sterile precautions. The morphology of these forms was similar to that of equivalent forms seen *in vivo* except for the occasional appearance of cytoplasmic spurs. The inoculation of these forms into chicks gave rise to a Type A e-e schizogony.

The tissue cultivation of avian e-e forms has been used to investigate the effect of drugs upon the e-e cycle. Tonkin (1946) investigated the efficacy of various drugs upon *in vitro* e-e forms of *P. gallinaceum*. Hawking (1947) and Hawking and Hunt (1948) used tissue cultures to demonstrate that proguanil itself is inactive and the active factor is a metabolite of proguanil. Scheng (1943) and Zuckerman (1946) incubated tissue slices and tissue emulsions containing e-e forms with various drugs for short periods to test their effect. Zuckerman was able to show that sulphadiazine is effective against e-e forms of *P. gallinaceum* even under such conditions. Knoppers (1949) has noted that a quinine-resistant strain of *P. gallinaceum* retained its resistance through the tissue cultivation of its e-e forms.

From the above it can be seen that all attempts to initiate e-e schizogony *in vitro* from erythrocytic forms have failed. It seems that there were three possible explanations for previous failures; two technical and one biologically fundamental.

It had been shown by Dubin and his colleagues that unless the macrophages of the culture are growing vigorously and for some time, they will not take up sporozoites, and pre-e forms could not develop *in vitro*. It seems, therefore, wise to allow spleen explants to grow for a few days before attempting infection of such a culture by means of erythrocytic merozoites.

All previous attempts at blood-form infection have been made with whole parasitized erythrocytes. Corradetti has shown that macrophages must be sensitized in some manner before they will engulf whole parasitized red cells. Naked parasites therefore might be more easily engulfed and subsequently grow. Repeated infections by such released parasites might sensitize the system to engulfment. There are some outstanding arguments against such a hypothesis but it seems worthy of investigation.

Both these requirements are easily fulfilled, but a further argument can be adduced for previous failures, which appears impossible to circumvent. Should successful infection of cultures be possible only by means of histiotropic merozoites produced by some host-parasite interrelation, this would prove an insuperable difficulty unless some mode of assuring a ready supply of such forms could be found. It is impossible in the present state of knowledge of merozoite tropisms to predict when the erythrocytic cycle may choose to produce histiotropic merozoites, even if such forms exist. Thus only the phagocytic factor can be taken into account in the initiation of e-e stages from erythrocytic stages.

An experiment designed to fulfil these conditions was carried out with the co-operation of Miss P. Vincent of the Wellcome Foundation, London. Explants of uninfected chick embryo spleen were set up on cover slips in Carrel flasks and allowed to grow for three days. Large numbers of naked erythrocytic forms of *P. gallinaceum* (obtained by saponin treatment) were then added to the culture. This was repeated three days later. All attempts to infect the explants were unsuccessful.

One major fault which can be eliminated in any further attempts was the clogging of the still cultures by parasite and red cell nuclei sludge. Roller-tube methods and the early replacement of the fluid medium should overcome this difficulty.

(e) GENERAL CONSIDERATIONS: RELATED GENERA

General Considerations

The questions of systematic classification arising from the consideration of various descriptions of e-e forms of malaria parasites will be discussed in the last chapter of this memoir.

The demonstration of the tissue development of the sporozoite has eliminated all doubt about the significance of the e-e cycle. It is an essential phase of the life cycle of the Plasmodiidae and it can no longer be held to be an accidental phenomenon arising from the blood phase and associated with some form of immunity breakdown.

Despite the confidence with which such a statement can be made and the assurance with which it is possible to discuss this tissue phase, there are a number of major lacunae in the knowledge of the e-e generations of avian and saurian malarias which it is worth while to tabulate here:

1. The pre-e forms of *P. elongatum* have yet to be described and the differentiation of this species from other species must depend to some degree upon the information yet to be gathered.
2. The mechanisms of change-over from tissue to blood or blood to tissue on the part of the parasite are most imperfectly understood. This knowledge is fundamental.

3. Further study upon sporozoite-induced e-e infections of *P. mexicanum* is necessary in order to determine to which pattern of e-e schizogony this parasite conforms.
4. Further knowledge of African saurian malarias would be useful apart from the reasonably conventional *P. pitmani* in view of the interesting stages of *P. agamae* described, which seem to show some similarities to *P. lacertiliae.*

Study of the e-e forms of the avian malaria of necessity raises the question posed by Manwell (1935): "How many species of avian malaria parasites are there?" There are, at present, about 29, which seem to be too many. The past tendency to multiply species while maintaining or even reducing genera and families is to be deplored. It is intended, however, to make only one appeal at present and that is to do away with *P. cathemerium* as a species distinct from *P. relictum.* All stages of both parasites and their host preferences are now known and the only accepted point of difference between the two species is the shape of the pigment granules in the gametocytes. It is my personal belief that the shape and size of pigment granules is liable to change not only with the parasite species but also with the host, the host cell and the state of immunity of the host. Not uncommonly workers have been unable to determine whether a parasite kept as a routine in laboratory birds is *P. relictum* or *P. cathemerium.* This conviction, that *P. cathemerium* and *P. relictum* are synonymous, has been crystallized by a recent verbal report made by Dr. M. D. Young. Dr. Young found that a parasite of wild birds which he was able to identify definitely as *P. relictum* took on the pigment characteristics of *P. cathemerium* when inoculated into canaries. Lastly, the variations from strain to strain of *P. relictum* are sufficiently great to encompass *P. cathemerium* as yet another strain of *P. relictum.*

Related Genera

No account of the e-e cycle of the avian malarias can be complete without some reference to the similar schizogony phase existing among the avian Haemoproteidae—*Leucocytozoön*, and *Haemoproteus*—where schizogony is exclusively exo-erythrocytic. It is not intended to indicate in any detail the life cycle of these parasites, or to consider species within these genera, as few morphological differences have been noted in the schizogony stages.

Leucocytozoön

Although Garnham (1953a) has suggested that a new family should be created to accommodate this genus, it is felt at present that, until the gametocyte's host cell is shown not to be an erythrocyte precursor, and while *elongatum* is kept in the Plasmodiidae, the genus must remain in the Haemoproteidae. The schizogony stages are not sufficiently unlike those of *H. vassali*, as demonstrated by Ray

(1949), or of other presumed *Hepatocystis* schizonts, to warrant the creation of another family.

The number of schizogony generations of these parasites is not known although they probably do not exceed three or four (Huff, 1942, 1949). The schizogony is transitory and cannot be found in birds displaying a mature erythrocytic infection. Except insofar as *Leucocytozoön* displays a relapse pattern (Mathis and Leger, 1909) the scarcity of schizogony forms would point to a minimum of generations, perhaps two.

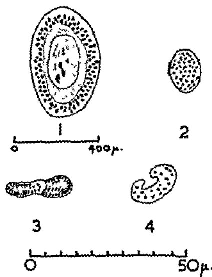


Fig. 5. Exo-erythrocytic schizogony of the avian Haemoproteidae in Section

1. Megaloschizont of *Leucocytozoön* in the spleen stained with Maximov's stain. 2. *One cytomere of Leucocytozoön stained with Maximov's stain.* 3. *Mature schizont* of *Haemoproteus* in the lung showing definite meroblastic formation and elongate merozoites. (Giemsa-colophonium.) 4. Immature schizont of *Haemoproteus* in the lung showing the peculiar nuclei seeming to have four segments and one nucleus apparently in early telophase. (Giemsa-colophonium.)

In the meantime it suffices to say that the tissue schizogony stages are found only early in the infection and generally only in young wild birds or during an epizoötic among domestic birds (Huff, 1942; Manwell, 1951b; Garnham (verbal communication)). I have searched unsuccessfully for these forms in rooks newly flown the nest and showing heavy infections of the blood.

Tissue schizogony stages of this genus were first postulated by Fantham (1910a) and he went on to describe such forms (Fantham, 1910a, 1910b). Unfortunately, the descriptions appear to involve some other parasite. Huff (1942)

gave the first and full description of the e-e forms of *Leucocytozoön*. He found that two types of schizogony occurred. Schizonts 11–18μ in diameter were found in parenchyma cells of the liver. Even at this size Huff was easily able to identify the parasite and the host cell. Megaloschizonts of up to 105μ were found in the spleen, heart, liver and intestine. Huff described bacilliform merozoites in the megaloschizonts and also a definite cytomere formation. Though there is little effect upon the parenchyma cell the megaloschizont caused tremendous hypertrophy of its host cell, the identity of which is uncertain. The host cell nucleus is also much enlarged but does not divide.

Wingstrand (1947, 1948) has found the megaloschizonts in the endocrine glands. He found that they measured up to 470μ in diameter. He was also able to show that their Feulgen reaction is similar to that of the plasmodia. Manwell (1951b) found megaloschizonts only in the kidney. I have studied sections of megaloschizonts in the spleen, kindly provided by Professor Garnham. The forms measure up to 500μ, but bacilliform nuclei were not seen (see fig. 5).

Laboratory transmission of *Leucocytozoön* has not been achieved either by arthropod vectors or by syringe transfer of the megaloschizonts. *Simulium* is generally accepted to be the insect vector. It seems at least possible that the megaloschizonts represent haemotropic merozoite-producing forms and pass through only one generation in each attack of the parasite. In this case the hepatic schizonts would produce histiotropic merozoites which, at a suitable time, initiate a new megaloschizogonic generation which, in turn, produces a relapse. This would follow the pattern apparently set in certain haemogregarines.

Haemoproteus

The schizogony of this genus is most commonly found in the vascular endothelium of the lung. Schizogony was first described by Aragão (1908) who decribed the elongated (up to 50μ), frequently twisted forms which typify the e-e forms which invade vascular endothelium. Acton and Knowles (1914) confirmed this discovery but also described young asexual forms of the parasite in red cells. Wasielewski and Wülker (1918) in an exhaustive study of the parasite confirmed Aragão's general observations but were unable to agree with Aragão's description of clear-cut cytomere formation. They believed each aggregation to be a separate schizont and that multiple infection of a single cell was therefore common. Wenyon (1926) and Huff (1942) held that the formations were true cytomeres. The present author has seen evidence of meroblastic formation but has not seen true cytomeres (see fig. 5). Wenyon (1926), however, has noted that cytomere formation is not the rule in some species and that while the majority of schizonts can be found in the lung, they are also to be found in the liver, kidney and spleen.

Sergent and Béquet (1914) and Coatney (1933) showed that no immunity is developed to the parasite. Coatney (1933) was successful in passaging the infection by emulsions of minced infected lung and showed that there exists no periodicity

of schizogony which obviously persists for a number of generations. Lastra and Coatney (1950) were able to show by means of blood inoculations and tissue transplants that the lung was infective up to 26 days after sporozoite infection while the spleen, liver, heart and brain were never infective. The blood was infective on the 4th day after infection. Lung was infective on the 14th, 20th and 28th days but not on the 4th, *8th and 12th days after sporozoite inoculation.* These figures allow no logical pattern, and no conclusions as to generation duration can be made.

The nuclear structure of the immature schizonts is interesting, as is shown in fig. 5. There is a distinct suggestion of early telophase stage in some nuclei. *The transmission of the parasite is cyclical in hippoboscid flies.*

CHAPTER 5

THE PRESENT STATE OF THE KNOWLEDGE OF THE EXO-ERYTHROCYTIC CYCLE OF THE MAMMALIAN PLASMODIA

(*a*) SPECIES: MORPHOLOGY AND CHARACTERISTICS

SOME knowledge of the pre-e or e-e cycles of 6 species of mammalian plasmodia is now available, but it is possible to guess the kind of cycle of many more.

The pre-e generation of schizogony has been identified in 2 simian and 3 human species:

P. cynomolgi	in *Macaca mulatta*	*cynomolgi* pattern
P. inui	in *Macaca mulatta*	*cynomolgi* pattern
P. vivax	in man	*cynomolgi* pattern
P. ovale	in man	*cynomolgi* pattern
P. falciparum	in man	

The e-e cycle has been identified only in *P. cynomolgi* infections, but an unidentified e-e stage of *P. knowlesi* has been described in *Macaca irus*. Various erroneous descriptions have been made which will be dealt with later.

Two patterns of e-e schizogony have been identified among these plasmodia by Garnham (1951a). These are the *cynomolgi* type and the *falciparum* type. It is intended to describe in detail the e-e cycle of *P. cynomolgi*, including personal observations. Comment upon other species will be made in relation to *P. cynomolgi*.

P. cynomolgi Mayer, 1907

The description of the pre-e cycle of this tertian parasite of macaques represented the first discovery of the long-sought tissue phase of mammalian plasmodia. The parasite presents certain convenient features which make it suitable for study. It displays the closest analogy to *P. vivax*, which was the parasite most studied for phenomena connected to a possible underlying tissue phase. *P. cynomolgi* is transmitted satisfactorily by numerous species of *Anopheles* which are indigenous at most centres of malaria research and are easily bred in the laboratory. The parasite produces a heavy but benign infection in the rhesus monkey, with high gametocyte counts. The infection persists for 6 months to 5 years.

The pre-e cycle was first announced by Shortt and Garnham (1948a) and Shortt, Garnham and Malamos (1948). These descriptions involved pre-e forms found 7 days after massive sporozoite inoculation. In later descriptions, Shortt and Garnham (1948b, 1948c, 1948d, 1948e, 1949) and Shortt (1948a, 1948b) were

to extend their discoveries to include pre-e forms found 5–10 days after sporozoite infection. An e-e form found 3½ months after infection was also described. These findings were immediately confirmed by Hawking (1948) and Hawking, Perry and Thurston (1948a, 1948b). These reports represented the discovery of relatively large plasmodial masses lying in the parenchyma cells of the liver of infected *Macaca mulatta* and were the result of very large numbers of inoculated sporozoites, probably not less than five million, from hundreds of mosquitoes.

The knowledge of this first generation of e-e schizogony was extended to include forms 2–17 days old, by the work of Shortt (1950, 1951a, 1951b), Shortt and Bray (1952), Shortt, Cooper and Bray (1953) and Shortt, Bray and Cooper (1954). As information concerning this phase is still being gathered, it is intended to split the descriptions of it into two parts, firstly taking the forms 4–14 days old, as they have been described, with additional personal observation. The descriptions will then be extended progressively and retrogressively in time to include those forms which I have played some part in uncovering. Thus the description can follow the history of research on this problem which makes the task of a reviewer easier and allows last-minute research to take its place in the text.

Huff (1948b), Huff and Coulston (1948) and Mulligan, Sommerville and Lloyd (1949) had failed in their attempts to uncover the pre-e stages of *P. cynomolgi* but in no case were more than 120 infected mosquitoes used or more than a few glands inoculated intravenously. In order to obtain results where others had failed, Shortt and Garnham commenced their experiments with not less than 1,000 *A. maculipennis atroparvus* so as to bring some 500 or so to the monkey at the critical time. Chiefly owing to the enormous numbers of sporozoites thus inoculated they were wholly successful in their search.

The 5-day-old pre-e schizont was seen by Shortt and Garnham to lie clearly in a parenchyma cell. Many other tissues had been examined but only the liver parenchyma was found to be infected. Accounts of the size and the number of nuclei of the 5-day-old form vary. Shortt and Garnham (1948c, 1948d), Shortt (1948a), and Garnham (1948b), have recorded an average diameter of 10·5μ and some 50 nuclei. Hawking (1948) and Hawking, Perry and Thurston (1948a, 1948b) gave an average diameter of 12 to 14μ and over 70 nuclei. I have measured only two 5-day-old forms and found them to measure 14μ in average diameter. However, on theoretical grounds, it is believed that the average diameter is in the region of 15μ and the number of nuclei from 50 to 100 (see fig. 16). At this size the parasite does not grossly enlarge the host cell, although the host cell nucleus is displaced to one side. The identity of the host cell is easily discernible.

The parasite tends to be slightly elongated at this age, presumably growing in the direction of some inherent weakness in the host cell and surrounding tissue. In those forms studied, this direction is apparently not towards the nearest sinusoid as might be expected. When stained by Shortt and Cooper's (1948) method of Giemsa stain and colophonium differentiation, the cytoplasm is typically

a pastel or darker blue depending on the degree of *differentiation. There are no* vacuoles in the cytoplasm, which frequently appears granular and sometimes shredded and tenuous. There is little tendency to the distinct cytoplasmic aggregation which characterizes later forms. The nuclei are red to purple, roughly spherical or oval, blocks having a slightly hazy outline. Rod shapes and bacilliform *shapes are rare.*

The parasite generally lies in a clear space which has been ascribed to differential shrinkage on fixation. While it is certainly true that the parasite and the host cell are of a very different consistency after fixation, as evidenced by their different reactions to the microtome blade, it is none the less possible that the parasite feeding as it must upon the host cell may be surrounded by a small clear space *in vivo.* Such a clear space is in evidence and is more noticeably regular about the *younger parasites and may be absent about the older forms.*

Garnham (1948b) finds that a characteristic of the 5-day-old parasite is a clear-cut margin. I have not had sufficient experience with this stage to challenge this view, but, on general evidence, am inclined to doubt it. A clear-cut margin is not a general occurrence among pre-e schizonts in sections ; 4-day-old forms have *been seen having fuzzy and irregular outlines ; the two 5-day-old parasites* examined have yielded no evidence of an envelope although one had what might be described as a clear-cut margin. The conclusion has been reached that the parasite in general has the most tenuous of envelopes and that the appearance of a well defined margin is a function of the histological treatment rather than any inherent character of the parasite or its stage of development. Two older schizonts have been seen to have a surrounding envelope of reasonable proportions and in each case it has been plain that this process was a product of the host cell cytoplasm. Some wisps of host cell cytoplasm remain clinging to the periphery of the parasite when it draws away from the host cell proper under the contracting influence of the fixative. These remains form a broken ring around the parasite, giving it the appearance of possessing a cell wall.

The 6-day-old pre-e parasite measures about 20–25μ in average diameter and may contain 200–500 nuclei. The parasite at this stage enlarges the host cell, which becomes merely an envelope about the parasite. The host cell nucleus is pushed completely to one side and may be slightly flattened. The cytoplasm of the parasite is light to dark blue and may be tenuous. Most often at this stage the *cytoplasm is plentiful, granular and often coagulated into strongly* basophilic aggregations 1–5μ in diameter. This may be a fixation phenomenon but more likely it represents a true clumping of ribonucleic acid, perhaps occurring in the hiatus between chromatin divisions. One or two vacuoles are often present in the cytoplasm. Curious roughly circular indentations also occasionally appear in the parasite *outline. These may be vacuoles burst on* handling or may represent true indentations, as is believed by Raffaele (1951). The contents of these vacuoles are unknown, but some reference to them will be made later. They vary in diameter

from 1 to 10μ. The number of these vacuoles is to some degree a measure of the health of the parasite, and a healthy 6-day-old pre-e schizont should probably not contain more than three large vacuoles. The significance of the vacuoles is unknown as they contain no particulate matter and the schizont cannot be observed in fresh preparations.

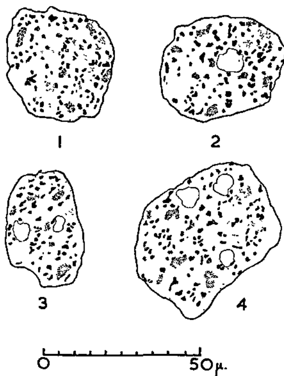


Fig. 6. Pre-erythrocytic schizonts of *P. cynomolgi* in liver sections. 7 days old. Stained with Giemsa-colophonium.

1. Solid schizont. 2. Schizont with 1 vacuole. 3. Schizont with 2 vacuoles. 4. Schizont with 3 vacuoles. Note the cytoplasmic aggregations and the complete irregularity of nuclear structure and distribution.

The nuclei may be spherical, oval, U- or V-shaped, branching, cubical or bacilliform in appearance. They often display an indistinct outline, possibly due to some impending division process, or possibly due to the depth of cytoplasm through which they are usually observed in sections 4 or 5μ thick. These nuclear shapes are best seen in the 7-day-old parasite The colour on staining varies from bright red to a reddish purple. The U and V shapes may have some mitotic significance; this seems doubtful, for, as will be shown, they are at this stage

Feulgen-negative. They seem to suggest a schizogonic binary fission of the nucleus; this could be interpreted as the formation of two chromosomes but this must remain very doubtful.

The parasite, as a whole, tends to some irregularity in shape, forsaking its previous spherical or ovoid shape; that is to say the parasite appears to depend to some degree upon outside stresses and strains to its rapid growth for the

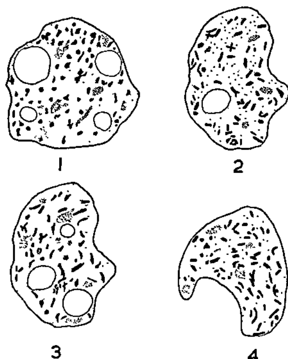


FIG. 7. Pre-erythrocytic schizonts of *P. cynomolgi* in liver sections. 7 days old. Stained with Giemsa differentiated in colophonium or acetic acid.

1. Schizont with 4 vacuoles (colophonium differentiation). 2. Schizont with 1 vacuole and bacilliform nuclei (colophonium differentiation). 3. Schizont with 3 vacuoles and bacilliform nuclei (colophonium differentiation). 4. Solid schizont with bacilliform nuclei (acetic acid differentiation).

direction of such growth. Taking the line of least resistance, it shows a tendency to push out blunt lobose arms in that direction. Certain indentations in the outline represent growth around Kupffer cells as the parasite grows out into a sinusoid. The host-cell nucleus also causes a small depression in the parasite shape. The host-cell nucleus itself undergoes no hypertrophy as in *Leucocytozoön* or multiplication as in *Hepatocystis*. Sometimes two host-cell nuclei can be seen in relation to a schizont. Since parenchyma cells with two nuclei are not uncommon in monkey liver this is no reason to suppose that proliferation of the host-cell

nucleus occurs. The surrounding tissue is slightly compressed but there is no reaction or infiltration in the area.

Most of the foregoing information remains true of the 7-day-old form (see figs. 6 and 7). This form is large, measuring an average of 36μ in diameter. This average is surprisingly constant from monkey to monkey over a large number of schizonts.

The cytoplasm of the parasite almost always displays aggregations of intense basophilia. The vacuoles may number up to 5 in healthy schizonts, the largest of which may measure 12μ across. Sometimes the cytoplasm may contain a larger number (15 or more) of minute vacuoles. The nuclei, numbering 1,000–3,000, may show all the forms mentioned above, particularly the bacilliform type (see fig. 7). Schizonts from one monkey may display all variations in shape of nuclei while in another they may all show a bar or bacilliform appearance. This bacilliform chromatin is not related to the age of the parasite and does not appear to be a certain stage in the whole schizogony process. It may be, however, a stage in each successive division of the chromatin throughout schizogony. If so, it would appear to be a pre-division stage equivalent to metaphase in mitosis. The finding of these forms in most schizonts in one monkey would in that case indicate a remarkable synchronism of chromatin division but this is, however, a more reasonable assumption than to postulate a host-dictation phenomenon with regard to the shape of parasite chromatin.

The parasite at this stage sometimes shows a different cutting reaction from the surrounding tissue, which becomes more pronounced with older forms. It appears that the fixed parasite matrix is more resistant to the knife and may pile up on the knife edge and cut in a wedge shape rather than at a consistent depth. The tail end of a schizont may be completely torn out of the surrounding tissue. This remains true of all of the e-e forms of the mammalian plasmodia and of the schizonts of *Nycteria*. The delicacy of the parasitic envelope is often evidenced at this stage when the parasitic matrix can sometimes be seen to have broken out and is escaping down a neighbouring sinusoid. Such a phenomenon occurring in association with an immature schizont can only be attributed to mechanical pressure due to handling, probably during removal of the tissue at autopsy or biopsy.

On the 8th day the first parasites are demonstrable in the blood. Hawking *et al.* (1948a) and Mulligan *et al.* (1949) showed that the blood becomes positive on subinoculation at this time. The present author has seen a tremendous flooding of the peripheral blood with tiny rings at 8 days after heavy sporozoite inoculation. On one occasion as many as 20 rings to a microscopic field were seen, but it is believed that this flooding is a measure of the number of merozoites produced in schizonts rather than a measure of the number of schizonts releasing merozoites after 8 days' growth. Although it is obvious that a number of tissue schizonts are releasing merozoites at this time, I have seen only one mature schizont containing merozoites at this stage. This observation was made among some 1,000

immature schizonts. Although the significance of this observation is tempered by the comparative rarity of visible mature schizonts at any time, it is believed that the number of *schizonts releasing merozoites on the* 8th day represents only 50 per cent. or less of the total number.

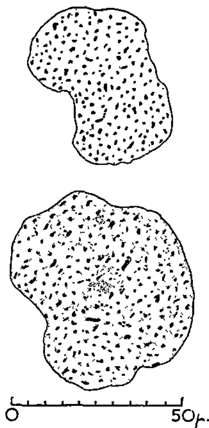


Fig. 8.

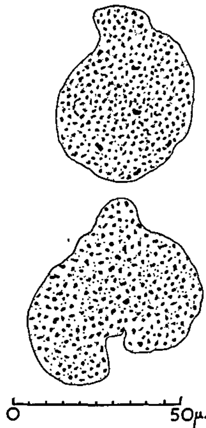


Fig. 9.

Fig. 8. Pre-erythrocytic schizonts of *P. cynomolgi* in liver sections. 8 days old. Stained with Giemsa-colophonium. Note the absence of vacuoles and the comparative lack of cytoplasmic aggregations.

Fig. 9. Pre-erythrocytic schizonts of *P. cynomolgi* in liver sections. 8 days old. Stained with Giemsa-colophonium.

The 8-day-old schizont represents a further simple growth stage from the 7-day-old parasite. It measures an average of 41μ across and now contains about *5,000 nuclei and possibly up to 10,000 (see figs. 8 and 9). This approximates* both in size and in numbers of nuclei to Grassi's (1900) estimate for oöcysts of *P. vivax* at the same stage of development. Hawking *et al.* (1948a) have reported an 8-day-old schizont of $68 \times 61\mu$. Nothing approaching this size has been seen since among

many hundreds of schizonts, and I am inclined to doubt the reported age of this parasite. The number of vacuoles has diminished and they are in fact more often absent, which indicates their disappearance prior to merozoite formation. Up to this stage there is an increase of nuclei per unit volume of parasite and on the 8th day they reach a high density.

The cytoplasm still displays aggregations and may display some cracks in its structure which can be interpreted as partially the result of fixation and probably to some degree a fleeting and vague attempt at meroblastic formation. The nuclei resemble those of the 7th day. What appears to be a true and very tenuous envelope can sometimes be discerned standing out from the parasite mass and enclosing a clear space of about 0·5μ in width around the parasite " endoplasm ".

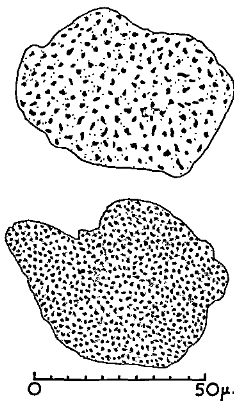
During the 8th and 9th days the majority of the schizonts (approximately 75 per cent.) come to maturity and the host parenchyma cell bursts. Something should be said about this process as it has been observed at times ranging from 8–21 days. At the stage of maturity these parasites measure 40–55μ as a rule, although exceptions up to 85μ have been seen late in the infection. Actual stages of merozoite formation such as can be seen in *P. inui* infections cannot be distinguished in maturing pre-e schizonts of *P. cynomolgi* A few parasites at this stage have been seen to display a cracking of the cytoplasm with nuclear arrangement about the fissures which approaches the almost cytomere formation of *P. inui* and *P. falciparum* pre-e schizonts but nothing more definite occurs (see figs. 14 and 19).

The formation of merozoites is obviously swift and haphazard. It appears to be a simple coalescence of cytoplasm about the nuclei without any amount of the patterning and budding off of nucleus-containing cytoplasmic processes such as is seen in the oöcyst and which by analogy may occur in *P. inui*. The release of formed merozoites may take rather longer to accomplish than the merozoite formation as more mature stages are seen than formative stages, although recognition of the formative stages is, of course, uncertain.

The merozoites themselves measure about 1–1·5μ. They are compact, consisting of a single nucleus and a compact piece of cytoplasm containing the suggestion of the vacuole which is to appear later in the erythrocytic ring stage. They stain an almost uniform red-purple and only after bursting and with critical lighting is it possible to discern the blue tinge in the cytoplasm. They lie in the schizont in a tightly packed haphazard manner and individual study is almost impossible.

The present author has seen only once the process of merozoite release from a schizont which had obviously occurred *in vivo* (see fig. 13). However, bursting *under mechanical pressure no doubt presents similar appearances. When the* schizont disintegrates the merozoites are released, largely into those surrounding sinusoids which border the parasite, these being the positions of least resistance to merozoite flow. They can be seen streaming down 2 or 3 interlinking sinusoids

and less often lying above, below and between cells in sections (see fig. 12). The great majority of these merozoites invade erythrocytes, where they can be seen as small delicate hair-like rings on the 8th and 9th days. This first erythrocytic trophozoite appears to be more delicate and the cytoplasm thinner than the later



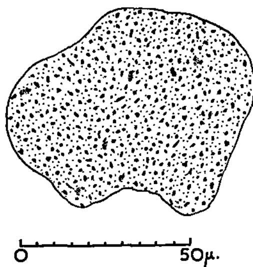


FIG. 10. FIG. 11.

FIG. 10. First generation exo-erythrocytic schizonts of *P. cynomolgi* in liver sections. 9 days old. Stained with Giemsa-colophonium.

FIG. 11. First generation exo-erythrocytic schizont of *P. cynomolgi* in liver section. 10 days old. Stained with Giemsa-colophonium.

forms derived from erythrocytic merozoites. A small minority of the merozoites are assumed to re-invade liver parenchyma cells (Shortt and Garnham, 1948e).

Some of the merozoites undoubtedly are engulfed by wandering macrophage cells and Kupffer cells, and equally undoubtedly perish there. Shortt and Garnham (1948c) have described infiltration of wandering macrophages into the area of a burst schizont, accompanied by active engulfment of the released merozoites. There are always some foci of macrophages in the liver for what appears to be no good reason. In only one of these foci have I identified merozoites (on the 8th

day). Also it should be said that unless the merozoites are still collected together it would be quite impossible to differentiate individuals from various fragments of liver tissue which are always present in sections. It is this fact which has discouraged me from attempting a search for merozoites re-invading or lodged in liver

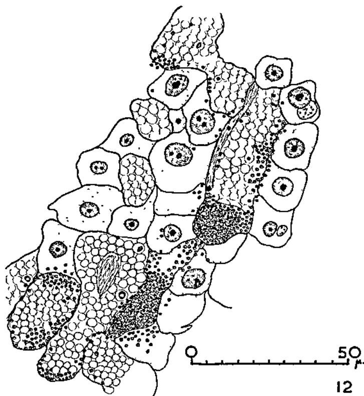
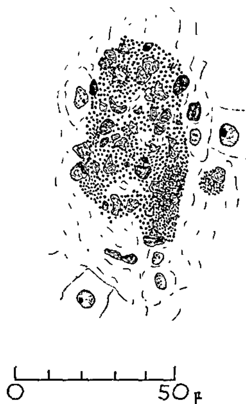


FIG. 12. First generation exo-erythrocytic schizont of *P. cynomolgi* bursting. 10 days old. Liver section stained with Giemsa-colophonium. Note the merozoites streaming down sinusoids and the prior establishment of an erythrocytic infection. The two aggregations of merozoites represent two lobose arms of a large schizont, the section being made through one end of it.

cells on the 10th or 11th days. Dispersal of merozoites from the area of the burst schizont is probably swift and it is certain that Shortt and Garnham's description of infiltration represents that type of observation which will be repeated only after very long search for just this phenomenon, unless the timing of the biopsy is exactly correct. (In my case after 3 years of intense search of innumerable preparations.)

On the 9th day after sporozoite infection those schizonts which are still maturing measure 40–50μ in diameter. The average of those measured is 46μ but, as schizonts have already released merozoites, an average figure is now an unreliable guide to either individual schizonts or to growth rate (see fig. 23). The



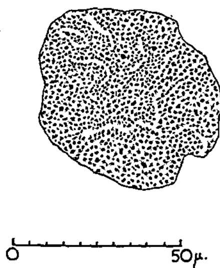


FIG. 13. FIG 14.

FIG. 13. Pre-erythrocytic schizont of *P. cynomolgi* ruptured. *8 days old. Liver* section stained with Giemsa-colophonium. Note the invasion by numerous wandering macrophages into the area of the ruptured schizont. The merozoites are still confined largely in the former schizont space.

FIG. 14. First generation exo-erythrocytic schizont of *P. cynomolgi* in liver section, 11 days old. Stained with Giemsa-colophonium. Note the cracking of the cytoplasm and the slight tendency towards meroblastic arrangement of the nuclei. This is the closest approximation to meroblast formation which *P. cynomolgi* achieves.

parasite appears to have between 5,000 and 10,000 nuclei. As a rule there are no vacuoles. In general they resemble 8-day-old forms (see fig. 10).

The immature forms on the 10th day measure an average of 51μ in diameter and resemble 9- and 8-day-old schizonts (see fig. 11); 11- and 12-day-old forms (see fig. 12) represent a further simple increase in size but have become rare compared with

the 8th-day forms. The actual drop in numbers appears to be of the order of 100 per cent. to 2 per cent. from 7 to 12 days after sporozoite inoculation. When working in conjunction with and under the direction of Professor Shortt, I have taken liver pieces from infected monkeys at 15, 17 and 105 days after infection and examined the tissue schizonts found in them. Details of this work have been published (Shortt, Bray and Cooper, 1954) and the 15-day-old form has been

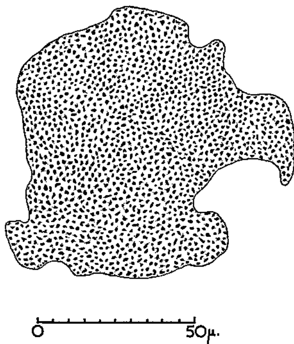


FIG. 15. First generation exo-erythrocytic schizont of *P. cynomolgi* in liver section, 15 days old. Stained with Giemsa-colophonium.

demonstrated (Shortt and Bray, 1952) and described (Shortt, 1951a). Although some description of the techniques involved in these experiments will be given later, it is convenient to describe these late primary schizonts now.

The 15-day-old first generation e-e forms seen were very large. One measured 108μ in its longest diameter and 71μ across. It was calculated by rather approximate methods to contain 60,000 nuclei. Another schizont measured $83\mu \times 75\mu$ and was estimated, somewhat more accurately, to contain some 40,000 nuclei. Neither of these forms was mature (see fig. 15). A mature schizont was seen at this time measuring $79\mu \times 62\mu$ and displaying, to a marked degree, the cracking of the matrix of the parasite. On the other hand, no arrangement of the merozoites

was noticed. This observation has led me to believe that cracking of the matrix of the parasite is not a very significant point, whereas nuclear arrangements have some significance, bearing in mind the fact that the mature oöcyst displays no arrangements of the formed sporozoites.

At this time, what were presumed to be second-generation schizonts made their appearance. These parasites were equivalent in size and morphology to 6-day-old pre-e schizonts. These forms were rare and in the light of the discovery of retarded forms to be described later it is no longer possible to be dogmatic about their origin. However, it is still thought that they do represent second-generation schizonts.

On the 17th day after sporozoite inoculation, one schizont measuring $65 \times 61\mu$ was seen. This parasite was considered to be of the first generation, for it was larger than any 9-day-old pre-e schizont recorded, which represents the greatest age a second-generation schizont could have attained. Some forms of 25–35μ in diameter were seen. At least one of these forms is believed to be a frank second-generation form on circumstantial evidence, as will be shown later.

It should be noted here that none of these smaller forms were seen to be collected together around the site of what might have been a burst first-generation schizont. This, however, is not considered to be any bar to their acceptance as second-generation parasites, as general immune reactions would be absent or nearly so, whereas specific infiltration would deal with localized merozoites about the area of release. It must be assumed that the merozoite destined to re-invade liver tissue takes a little time to do so.

These late first-generation schizonts cause considerable compression of the surrounding tissue but no reaction in it. The host-cell nucleus, though sometimes flattened and always pushed to one side, is otherwise unaltered. There is no piling up of wandering macrophages in the area due to capillary blockage as has been suggested by Raffaele (1951) and blood flow in the area appears normal. At this time (17–19 days) a new flooding effect in the blood can be seen if the primary blood infection is suppressed by division inhibiting agencies such as a milk diet. A wave of tiny rings occurs on about the 18th day (Bray and Garnham, 1953a).

Two late e-e forms of *P. cynomolgi* have been described by Shortt and Garnham (1948e) 102 days after sporozoite inoculation, just prior to a parasitic relapse in the blood. These forms were found in liver parenchyma and were rare compared with the pre-e forms seen in the same infection; 412 sections were examined to reveal these two parasites. I have examined 80 sections 105 days after sporozoite inoculation and one day after relapse to find two more late e-e forms (Shortt, Bray and Cooper, 1954). On the 7th day after infection this same monkey had shown 8 schizonts to a section. This represents a ratio of approximately 100 to 1. It is obvious therefore that by a combination of circumstances, including sensitized immune action against histiocytic merozoites and the small

proportion of tissue invasive merozoites, the number of tissue schizonts drops sharply after the pre-e generation. Succeeding generations in natural infections must be of a very low order (*cf. Leucocytozoön*).

These late e-e schizonts with one exception are morphologically identical with pre-e schizonts of similar size. They resemble 5-, 8- and 10-day pre-e forms. *The exception is completely atypical. It seems to be about 90–100μ in length as* it appears in 19 sections 5μ thick. It never measures more than 24μ in width and usually about 10μ. It has at least one and sometimes two arms for half its length. It follows the course of one, two or three sinusoids in the manner of a large e-e schizont of *P. gallinaceum* in a brain capillary. Apparently the schizont had grown out into a sinusoid and followed its course taking with it the parenchyma host cell. It is perhaps surprising that more pre-e forms do not follow a similar course, as a sinusoid would seem to be a line of little resistance to the parasite's rapid growth.

In 1949, taking the 5-day-old pre-e forms as a starting point, Professor Shortt laid plans to investigate the earlier forms, working back a day at a time with each successful experiment. I was to join this investigation after the discovery of the 4-day-old form (Shortt and Garnham, 1949). This form was found by Shortt to measure 10·2μ in its longest diameter and to contain 24 nuclei (Shortt and Garnham, 1949; Shortt, Bray and Cooper, 1954). Similar forms which I have seen have measured an average of 11μ in diameter and contained 32 to 36 nuclei. Further references to 4-day-old forms will be made (see also fig. 16).

It was the 3-day-old pre-e schizont which occupied so much time and entailed so much experimental work. As the details of the experimental methods have been published (Shortt, Cooper and Bray, 1954) and, as credit for the results belongs largely to Professor Shortt, the descriptions here will be confined to the morphology of the forms found and only the barest indication of the methods used will be offered.

Sporozoites were obtained from very large numbers of *A. maculipennis atroparvus*, varying in number from 120 to 6,700. The sporozoites were released by biting, gland dissection or crushing of thoraces or whole mosquitoes. The sporozoites thus obtained were introduced into monkeys by biting, or by syringe intramuscularly, intraperitoneally, subcutaneously, intravenously, into the hepatic portal vein or directly into exposed liver tissue. Liver pieces were taken by biopsy on open laparotomy 2 or 3 days after sporozoite infection. More liver was taken at 7 or 8 days after infection by biopsy or autopsy as a check on the measure of infection. It was found that any number of 7- or 8-day-old schizonts less than 2,000 per cm.^{3} of liver tissue spelt failure of attempts to find reasonable numbers *of early forms. The most successful experiment in which 2-day-old forms* were discovered at a rate of *2* per section was one in which some *25,000* schizonts per cm.^{3} were found on the 7th day.

The 3-day-old schizonts demonstrated by Shortt (1951b) measure 5μ ± 1μ in diameter and lie in a parenchyma cell. The parasite lies at the periphery of the

2. They may represent true arrested and latent schizonts, as have been suggested by some workers to be the causative organisms of long-term relapses. (Boyd, 1953 ; Shute, 1953).
3. They may represent normally growing schizonts arising from sporozoites delayed 1, 2, 3 or 4 days on their journey to the liver. Such a delay might

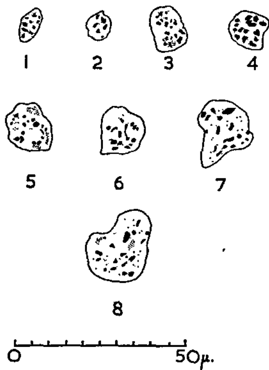


FIG. 17. "Retarded" pre-erythrocytic schizonts of *P. cynomolgi* in liver sections. 7 days old. Stained with Giemsa-colophonium or Giemsa-acetic acid. All schizonts are 7 days old but are of the size of normal schizonts of earlier days.

1. 3½-days-old size } same schizont.
2. 3½-days-old size }
3. 4-days-old size.
4. 4-days-old size.
5. 4½-days-old size } same schizont.
6. 4½-days-old size }
7. 5-days-old size.
8. 6-days-old size.

be occasioned by a journey via the lymph system or by fruitless sojourning in interstitial spaces of some cell system not particularly well supplied with active macrophage cells. Alternatively a few sporozoites may remain latent for similar periods in liver parenchyma cells.

4. They may represent more slowly growing schizonts derived from sporozoites which gained liver cells at the same time as other sporozoites. That is to say that, though they appear retarded they are not to be con-

sidered abnormal and that some slow growth should be considered typical for a small proportion of the pre-e schizonts. Such a faculty would not represent a sharply distinct manner of growth but run the gamut from near cessation to just sub-normal rates.

On the first suggestion, I believe that the demonstration of the 2-day-old pre-e trophozoite eliminates any possibility of two pre-e generations in *P. cynomolgi*, and I therefore reject this suggestion.

On the second suggestion, there is no evidence to prove definitely that *P. cynomolgi* displays a long-term relapse pattern. On the contrary, Schmidt and Genther (1953) and Schmidt (1953) hold that the pattern is the short-term South-West Pacific *vivax* type. Thus the latent schizont postulation which has been advanced to explain long-term relapses may not apply to *P. cynomolgi*. This, however, does not disprove the theory. There is nothing about these forms which leads one to believe they have completely ceased to grow. Some nuclei are in the state which has been accepted as active in some undefined sense. There is absolutely no cyst wall or any other suggestion of latency. Lastly, the "retarded" forms seen on the 10th day are larger than those seen on the 7th day and are believed to represent active growth of the earlier forms.

The third theory is less easily disposed of than the two above. Sporozoites may remain viable in the mosquito's glands for months and in blood *in vitro* for up to 5 days. There is no reason to suppose that a sporozoite may not live for days in the vertebrate host if not attacked. The fact that the blood is consistently negative from one hour to 8 days after sporozoite inoculation tells against any late comers reaching the liver by means of the blood stream, though there are loopholes in this demonstration, as the eqivalent numbers necessary to produce the "retarded" forms seen in the present series might not have been sufficient to set up an infection on inoculation in the experiments of Mulligan *et al.* (1949). The lymphatic route which has been suggested by Griffiths and Gordon (1953) and Lloyd and Sommerville (1949) after mosquito bite or intradermal inoculation is discounted in the present descriptions, as all "retarded" forms have been seen after intravenous inoculation of sporozoites. Sporozoites have been reported in a lymph node 24 hours after subcutaneous inoculation, by Boyd and Kitchen (1939), but it is not accepted that sporozoites would specifically enter the lymph after intravenous inoculation. On the other hand, on intravenous inoculation such as was used it is possible to postulate some sporozoite retention in and late release from mosquito gland debris caught up in lung capillaries, although it seems that such material would be rapidly dealt with by infiltration and vascular endothelium which acts against mosquito debris remarkably quickly. Thus, although this general theory cannot be definitely rejected, it presents too many difficulties when considered beside the last postulation.

The explanation of choice is the last, chiefly because it is both simple and obvious. A similar type of irregular growth is exactly paralleled in the sporo-

2. They may represent true arrested and latent schizonts, as have been suggested by some workers to be the causative organisms of long-term relapses. (Boyd, 1953; Shute, 1953).
3. They may represent normally growing schizonts arising from sporozoites delayed 1, 2, 3 or 4 days on their journey to the liver. Such a delay might

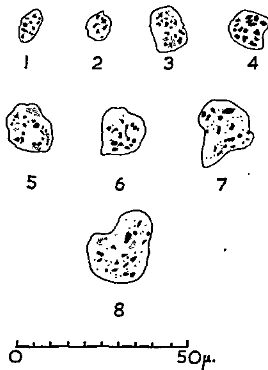


FIG. 17. "Retarded" pre-erythrocytic schizonts of *P. cynomolgi* in liver sections. 7 days old. Stained with Giemsa-colophonium or Giemsa-acetic acid. All schizonts are 7 days old but are of the size of normal schizonts of earlier days.

1. 3½-days-old size } same schizont.
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3. 4-days-old size.
4. 4-days-old size.
5. 4½-days-old size } same schizont.
6. 4½-days-old size }
7. 5-days-old size.
8. 6-days-old size.

be occasioned by a journey via the lymph system or by *fruitless sojourning* in interstitial spaces of some cell system not particularly well supplied with active macrophage cells. Alternatively a few sporozoites may remain latent for similar periods in liver parenchyma cells.

4. They may represent more slowly growing schizonts derived from sporozoites which gained liver cells at the same time as other sporozoites. That is to say that, though they appear retarded they are not to be con-

136		
Day 0		140 infected *A. maculipennis* were allowed to feed; 98 mosquitoes fed.
Day +8		Liver exposed by laparotomy; piece of liver excised.
137		
Day −2		Starved as above.
Day −1		Starved. Phlorhizin-treated as above.
Day 0	11.00 a.m.	Phlorhizin-treated as above.
	3.15 p.m.	Liver exposed by laparotomy; 160 infected glands inoculated as above.
Day +1	3.15 p.m.	Died under anaesthesia. Inoculated area excised.
138		
Day 0		Infected glands of 98 *A. maculipennis* inoculated intravenously.
Day +4		Liver exposed by laparotomy. Piece of liver excised from the left lobe.
Day +8		Liver exposed by laparotomy. Piece of liver excised from the right upper lobe.
Day +10		Liver exposed by laparotomy. Piece of liver excised from the right lower lobe.
Day +21		Died as a result of the infection despite quinine. Various pieces of liver taken.

All excised liver pieces were fixed in Carnoy's fluid, dehydrated, embedded nd sectioned at 3 or 4μ thickness. Sections were stained with Giemsa-colophonum, Feulgen, Gram's stain or the alkaline phosphatase method of Danielli. Two echniques were used to obtain sporozoites for inoculation by syringe. In each ase the supporting medium was 15% inactivated monkey serum in Locke's luid, chilled where possible to 5°C. as recommended by Brackett and Hughes 1945) and Porter, Laird and Dusseau (1952).

The technique used for the infection of M138 is routine in this laboratory and was first demonstrated by Garnham (1951b) and has since been described by him in more detail (Garnham, 1954). It involved the dissection of the glands from prepared mosquitoes by numerous dissectors at the same time. The glands are picked up in a drop of medium in a Pasteur pipette and transferred to a receptacle. All glands are eventually pooled, lightly ground and inoculated intravenously. For the infection of M135 and M137 the maximum number of sporozoites was required in the minimum amount of fluid. The following modification to Garnham's method was therefore adopted. Perspex slides were constructed having a depression ¼ inch deep at one end. Two drops of medium were placed in this depression and covered with a cover slip. One drop of Locke's fluid was placed on the slide. The glands were dissected out dry, picked up on the point of a needle, rapidly moistened in the Locke's fluid and transferred to the medium. The cover slip was removed and the glands scraped off the needle into the medium in the depression, with rough handling to break them up. By this means 160 glands were collected in 8 drops of fluid by four workers in ¾ hour. After a little

gonous cycle. The lack of synchronism in the release of merozoites from the pre-e cycle is well established. The "tail off" of the growth-rate curve of the pre-e forms (see fig. 21) is also taken to mean that as normal forms release merozoites and disappear, so the remaining "retarded" forms reaching maturity reduce the average size of the late forms. A similar phenomenon is also apparent in the sporogonic stages. Since asynchronism is normal for the process of merozoite formation and release there is no reason to believe that asynchronism of growth does not also occur. For these reasons this last explanation is adopted and certain other postulations will be based upon it later in this work.

It is now intended to detail an experiment recently undertaken to demonstrate the 24-hours and 6-hours-old pre-e trophozoites of *P. cynomolgi*. Although largely unsuccessful it is detailed here to demonstrate the general techniques used in this type of investigation. In addition to the main object, work was done to obtain e-e forms 21 days after sporozoite inoculation and to obtain material at 3, 4, 8, 9 and 10 days after infection for further study. Some of the information gathered has already been incorporated in the descriptions above. Shortt, Bray and Cooper (1954) have said that all that remains to be done in order to obtain a complete series in one mammalian plasmodium is to describe a 24-hours-old pre-e form of *P. cynomolgi*. The present experiment was designed to fulfil this obligation and also elucidate the immediate fate of the sporozoite. In addition, it was considered necessary (on the suggestion of Professor Garnham) to make a comparison of the efficiency of the natural mosquito bite mode of sporozoite inoculation and those methods of sporozoite introduction routinely used in these experiments.

Materials and Methods

Four *Macaca mulatta* were set aside and numbered M135, M136, M137, and M138. Approximately 1,000 *A. maculipennis* were infected with *P. cynomolgi* by feeding on a suitable gametocyte donor ; 12 days later the surviving mosquitoes, numbering about 700, showed a sporozoite rate of 85% with heavy gland infections. The monkeys were put on the following regimens :

M135

Day −2		Deprived of all solid food and given water only.
Day −1		Starved. Inoculated intramuscularly with 100 mgm. phlorhizin in propylene glycol per kgm. body weight.
Day 0	8.00 a.m.	Phlorhizin-treated as above. Starved.
	11.45 a.m.	Liver exposed by laparotomy. *160 infected A. maculipennis glands taken up into a lumbar* puncture needle and inoculated into the right upper liver lobe tip. The area marked by cautery.
	5.45 p.m.	Liver exposed by laparotomy ; inoculated area excised.
	6.00 p.m.	Returned to normal diet.
Day +3	11.00 a.m.	Liver exposed by laparotomy ; liver area bordering one side of the inoculated area excised.
Day +9	11.00 a.m.	Sacrificed. Liver area bordering the other side of the inoculated area excised.

Very large numbers of 8-day-old pre-e forms were seen in M138 intravenously infected with sporozoites, while very few were seen in M136 infected by bites. The number of schizonts found in M138 was 3,500 per cm.^{3} of tissue. The number found in M136 was 64 per cm.^{3} of tissue. *This represents a ratio of 55 : 1.* None of the 8-day-old forms seen was mature.

A maturing schizont seen on the 21st day is shown in fig. 18. It proved impossible to tell whether this form was of the first or second generation.

The main purpose of the experiment was the search for the early forms. After direct inoculation various artefacts appeared which made the task of the searcher more difficult than usual. Some bacteria and some spores were seen and considerable infiltration and debris was collected around the site of inoculation which was made obvious by the easily visible haemorrhage in the path of the needle. Unfortunately the chief object was not definitely achieved. Many bodies were seen resembling the 2-day-old stage and the mental picture the author had of the earlier forms. However, each body displayed some essential difference from the appearance of a correctly stained parasite. After long search one body in sections of liver taken at 24 hours was found which seemed to fit the criteria. It was about 2μ in diameter, had a blue cytoplasm mostly collected to the periphery with a suggestion of a vacuole. The body as a whole was spherical with some distortion towards the triangular. It lay apparently in a parenchyma cell. Only the nucleus of the body was unsatisfactory. This was a smooth sphere of red to purple staining. It was felt that the nucleus would be red and irregular. However, the body resembles none of the spores seen in the same section.

A similar body was found in the liver taken 6 hours after inoculation. This finding has tended to discount the previous finding as, although this body was ovoid, it seemed too far developed from a sporozoite considering the fact that it had had only 6 hours for that development. In all other aspects it resembled the first body.

Discussion

The comparison of the efficiency of the two modes of inoculation is interesting. If it is assumed that as little as 10% of the sporozoite content of the glands is inoculated on biting, this still does not account for the figure of 1·8 per cent. reaching the liver compared with intravenously inoculated sporozoites. Undoubtedly a proportion of mosquito-inoculated sporozoites are taken back up into the gut of the mosquito as it feeds on the blood pool, but this still would not explain such a low figure. As both series of sporozoites must run the gauntlet of the general lymphoid-macrophage system it must be assumed that a very considerable proportion of the mosquito-inoculated sporozoites are lost in the skin. This may be due to *local* phagocytic activity, enmeshment *in local tissue or the taking of* the wrong turning such as a fruitless wandering in the lymphatics.

One of the surprising features seen was the rapidity and efficiency the live

practice almost no chitin accompanied the glands into the medium. The slide was chilled periodically by contact with ice. The concentration of sporozoites achieved was extraordinarily high and some 2×10^6 sporozoites were calculated to be free in the 8 drops of fluid. The fluid and sporozoites were taken up into the barrel of a lumbar puncture needle, the intake being controlled under the microscope to ensure the complete uptake of all liquid and particulate matter. All mosquitoes came from the same batch and all were infected simultaneously from

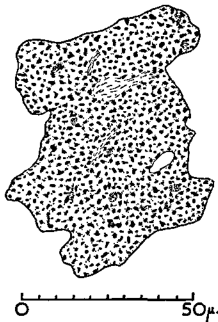


FIG. 18. Exo-erythrocytic schizont of *P. cynomolgi* in liver section found 21 days after sporozoite inoculation. Stained with Giemsa-colophonium. It is impossible to say whether this schizont is first or second generation. There is some cracking of the cytoplasm.

the one monkey to ensure a constant infection in various subsequently separated batches.

Results

First-generation e-e schizonts were seen on the 4th, 8th, 9th and 10th days after infection without undue microscopic labour. Two schizonts 4 days old were found. They measured $13\mu \times 9\mu$ and $14\mu \times 8\mu$. In one form the nuclei were largely collected to one side of the parasite. In both parasites the cytoplasm was very tenuous. Both are shown in fig. 16. Normal 9-day-old and 10-day-old schizonts were seen. The liver piece taken 10 days after infection revealed the merozoite releasing schizont shown in fig. 12. It will be seen that the erythrocytic infection is already established.

been cauterized to prevent haemorrhage, the picture on the 9th day was quite striking in that it presented a line of schizonts running down the border of the cauterized tissue leaving the remainder of the tissue uninfected.

The distribution of schizonts in the liver after intravenous inoculation of sporozoites is apparently haphazard although there appears to be a tendency towards relative aggregations of 4 or 5 schizonts in a comparatively small area of section. This may be due to tangled sporozoites becoming separated only at the last moments of penetration into sinusoids.

P. inui Halberstädter and Prowazek, 1907

The pre-e forms of this quartan parasite have been studied by Garnham (1951b) in the liver of *M. mulatta*. He has given details of the forms 7, 8, 11 and 12 days old and, by his courtesy, the author has had an opportunity of studying the merozoite formation process of this parasite when 10, 11 and 12 days old. It is with Professor Garnham's kind permission that some unpublished observations on this process are included here. He was successful in transmitting this parasite through *A. maculipennis atroparvus*, introducing sporozoites from these mosquitoes in large numbers intravenously into *M. mulatta*.

The 7-day-old pre-e schizont is spherical, 6–8μ in diameter and contains only 5 to 10 nuclei. The effect upon the host parenchyma cell is almost negligible The cytoplasm is patchy and rather tenuous, resembling that of its close equivalents in size, the 3- and 4-day-old pre-e schizonts of *P. cynomolgi*. The nuclei are more numerous and more regular in outline than those of *P. cynomolgi*. The stain reactions are similar except for a somewhat greater basophilic reaction of the cytoplasm although this may be due to differences in the extent of colophony differentiation.

The 8-day-old form is spherical, 10–11·5μ in diameter with 25–30 nuclei and a rather attenuated cytoplasm. The nuclei are regular in shape. The host cell nucleus is somewhat displaced but there is little hypertrophy of the parenchyma cell as a whole (see fig. 19).

The 11-day-old form is 16–20μ in diameter and roughly spherical. Condensed cytoplasmic aggregations are present as in the older pre-e schizonts of *P. cynomolgi*. No vacuoles are present and the parasite in general displays a much more orderly appearance than the 7- and 8-day-old pre-e forms of *P. cynomolgi*. The growth rate, as can be seen, is much slower and this seems to make for a tidier and more patterned method of growth. Some meroblastic nuclear arrangement can be discerned (see fig. 19).

The 12-day-old form is generally mature, and in fact recent experiments have shown that merozoite formation commences on the 10th day and is often complete on the 11th day. The mature schizont is 20–24μ in diameter, though extremes of greater or less diameter occur. It is usually roughly spherical or ovoid. The schizont contains an estimated 2,217 merozoites of 1μ in diameter, frequently

displayed in dealing with the large mass of gland tissue inoculated. Within 24 hours such inoculated tissue was quite unrecognizable and the majority of it was cleared. At the same time the infiltration and inflammatory reaction was localized to about 1 mm. on either side of the needle path. Even at 6 hours after inoculation much of the debris had been cleared and gland tissue was largely unrecognizable. The resultant artefacts in the liver are worthy of note. Coulston (1949) and Coulston and Robinson (1950) have described pre-e forms of *P. cynomolgi* in R-E cells of the liver 47 and 89 hours after direct inoculation of 18 and 24 ground thoraces of infected mosquitoes respectively. These are described as measuring 12–18μ in diameter. Bodies were seen in the present experiment closely resembling these descriptions. They varied in diameter from 3 to 20μ and displayed a very close similarity to immature forms of *P. gallinaceum* in liver sections when stained with Maximow's stain or when poorly differentiated after Giemsa staining. They show minute vacuolation and lighter areas which resemble badly stained nuclei. They were comparatively numerous and it is felt that, as Coulston used Maximow stain routinely, these bodies are what he has described as pre-e forms. However, that they are not parasites is quite obvious. They display a dark grey-blue appearance with no red nuclei in well differentiated sections, which is typical of certain other artefacts and is a point of differentiation from pre-e schizonts. Similar material can be seen lying about the section free in streaks, particularly in the area of haemorrhage where no fixed tissue cells can be found. Lastly and *conclusively, all dimensions of these bodies can be seen both 6 hours and 24 hours* after inoculation and, by no stretch of the imagination can a pre-e schizont developing from a sporozoite be thought to reach the size of 20μ in diameter in 6 hours. *In fact, the bodies are engulfed debris from the inoculation.* They are not visible 8 or 9 days after direct inoculation of glands.

At present it has been decided that the 2 bodies described above, 6 and 24 hours after sporozoite inoculation, represent a rare spore of vegetable origin which differs in staining reaction from other spores seen.

One last point should be mentioned on the subject of *P. cynomolgi* which concerns the distribution of pre-e schizonts in the liver. After direct inoculation of sporozoites into the liver the schizonts are quite definitely localized. On one occasion one lobe of the liver was inoculated with 120 glands from heavily infected mosquitoes by Professor Shortt and myself. Some 2,000 schizonts per cm.^{3} were found at the site of inoculation but none was found in other lobes on the 7th day after infection. On the 17th day after infection small schizonts were found in the inoculated lobe but, in addition, one small schizont was found in another lobe and was for this reason thought to belong definitely to the second generation. In the experiments described above where 160 infected glands were inoculated into a small portion of one lobe, schizonts found on the 9th day in the piece of tissue *adjoining the inoculated area were confined to that side* of the sections which had adjoined the inoculated area. As the previous excision of the inoculated area had

lack of synchronism as *P. cynomolgi*. Though this 8-day incubation is the rule, Raffaele has frequently referred to finding the blood positive on the 5th day after sporozoite inoculation. De Sanctis Monaldi and Raffaele (1953) have recently published this information and Raffaele (1946) has referred to the phenomenon as *belonging to the Italian strain of P. vivax. This may then be a strain characteristic.*

Because of repeated observations on the radical cure of blood-inoculated *P. vivax* infections as compared with the lack of efficacy in schizontocidal therapy of sporozoite-induced cases it has always been assumed that the underlying tissue phases of *P. vivax* cannot be initiated by erythrocytic forms. No observations have been made to alter this decision and no similar pre-e stages have been found in blood-inoculated infections in *P. cynomolgi* (Corradetti and Verolini, 1950, 1951). Similar chemotherapeutic observations have been made on *P. cynomolgi* and *P. malariae*. It can be said with certainty then that the e-e cycle in mammalian malaria cannot be initiated by blood parasites, leaving aside for the present the doubtful cases of *P. berghei* and *P. vinckei*. Hawking and Thurston (1952) failed to obtain development of *P. vivax* on direct inoculation of sporozoites into the liver of *M. mulatta*. Huff and Coulston (1948) reported a pre-e form under similar conditions, but this description is to be discounted.

P. ovale Stephens, 1922

The pre-e cycle of *P. ovale* has been demonstrated recently by Garnham, Bray, Cooper, Lainson, Awad and Williamson (1954a, 1954b, 1955).

The strain of *P. ovale* which originated from Liberia was maintained among the workers themselves, which has caused them to view with some disfavour those textbooks which refer to *P. ovale* as causing only a mild disease. This work stands as a monument to the courage and selflessness of Mr. W. Cooper, Class Demonstrator of the Department of Parasitology, London School of Hygiene and Tropical Medicine, who volunteered to undergo the necessary laparotomy and biopsy of a large segment of liver. He must be the only man who has sectioned, stained and examined large amounts of his own liver and expressed great pleasure at the foreign bodies he found there. The liver biopsy was performed 9 days after the first of 3 days of sporozoite infection which was effected by the bites of 750 infected mosquitoes. Scanty pre-e forms were found of sizes probably equivalent to fifth day and 8- or 9-day-old schizonts. The presumed 5-day-old forms are *typical immature* mammalian pre-e forms of about 40μ in diameter. They are usually oval with lobulated contours. The most striking aspect is the relatively immense nuclei of about 2μ in diameter. The cytoplasm frequently contains a number of clefts but no vacuoles of the *P. cynomolgi* type.

The 8- or 9-day-old forms show a superficial resemblance to the 5- or 6-day-old pre-e schizonts of *P. falciparum*.

They are large, of up to 60 and 70μ in diameter, and of a highly lobulated

arranged in meroblasts (see fig. 19). There is some hypertrophy of the host cell but no reaction in the surrounding tissue. In general the parasite differs from the equivalent *P. cynomolgi* schizont in having definite meroblastic arrangement of the cytoplasm and nuclei or of the merozoites and in having a more tenuous cytoplasm. It takes about 3 days longer to mature, which is proportionally somewhat less than the equivalent comparison in sporogony times of *P. vivax* and *P. malariae*. A rough graph of the growth rate indicates a long maturation period of the sporozoite which probably takes 3 to 4 days to round up and commence to divide. This would parallel the behaviour of the young oöcyst in *P. malariae* infections (see fig. 21).

The merozoite formation process of *P. inui* is most interesting. On or about the 10th day after sporozoite infection the parasite begins to display what Garnham (1951b) has called pseudo-cytomere formation. The parasite matrix breaks up into individual masses of cytoplasm containing the nuclei with small twisted clear spaces between the masses. These masses, which the author has preferred to call meroblasts, commence final stages of merozoite formation, possibly with one further nuclear division, by a process probably similar to that of sporogony. That is, blunt fingers of cytoplasm protrude from the septa, carrying with them the nuclei, and finally break off. On the other hand, there is at present no evidence to show that the cytoplasm does not simply break up and coalesce about the individual nuclei. The formed merozoites often stay in the meroblastic patterns or frequently migrate to the periphery to form a palisade effect. The full process is not yet clear, but meroblastic formation equivalent to the septa formation in the oöcyst is the prime feature.

P. vivax Grassi and Feletti, 1890

The pre-e cycle of *P. vivax* was foreshadowed by the work on *P. cynomolgi*, and later in 1948 Shortt, Garnham, Covell and Shute (1948) announced its discovery. The pre-e forms were found in the liver of a human volunteer 6 and 7 days after heavy sporozoite infection. Full details of the work were published and demonstrated by Shortt and Garnham (1948c, 1948d). Sporozoites from 2,010 *A. maculipennis atroparvus* were introduced by biting into a human volunteer over two days. Liver biopsy was performed 6 days after the last day of biting. The volunteer had been therapeutically infected with *P. vivax* 22½ months previously, by inoculation of blood forms, and after the second and apparently overwhelming infection displayed no parasitaemia or fever.

The pre-e forms found measured 42μ in the longest diameter and were thought to contain 1,000 nuclei. It seems certain that this estimate is low and the figure is probably closer to 10,000. The forms were found to resemble in every respect the equivalent forms of *P. cynomolgi* (see fig. 19). The duration of the pre-e cycle is the same as that of *P. cynomolgi*, and Fairley (1947) has shown that the blood becomes positive upon the 8th day. No doubt *P. vivax* displays the same

several thousand mosquitoes were used and when a large piece of liver was taken by open abdominal operation was success obtained.

The 3-day-old pre-e parasite measures some 20μ in diameter and contains 40 or more nuclei (Jeffery *et al.*, 1952). The nuclei are rather larger than those of *P.*

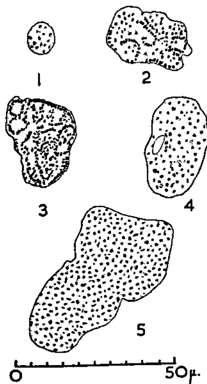


FIG. 19. Pre-erythrocytic schizonts of various mammalian plasmodia in liver sections. Stained with Giemsa-colophonium.

1. 7-day-old pre-e schizont of *P. inui*. 2. 11-day-old pre-e schizont of *P. inui*. Note the beginning of meroblastic arrangement. 3. 12-day-old pre-e schizont of *P. inui*. Note the meroblastic formation and palisade effect. Note also the comparative regularity of the nuclei. 4. 7-day-old pre-e schizont of *P. vivax*. This section does not show the widest diameter of the schizont. 5. 5-day-old pre-e schizont of *P. falciparum*.

cynomolgi at a comparable size though perhaps not at a comparable age. The cytoplasm is similar to that of 5-day-old forms of *P. cynomolgi*.

The 4-day-old form is similar and measures 30μ in diameter. It probably contains 1,000 or more smaller nuclei. It can be seen that the rate of growth and division is very fast indeed.

contour. The immature schizonts have a denser display of nuclei which are smaller than those of the 5-day-old forms. The nuclei are frequently arranged peripherally and are Feulgen-positive.

The formation of merozoites was well illustrated in this parasite and showed clearly a process of which *P. cynomolgi* had given hints previously. The merozoite formation begins at the extremities of the parasite, that is the first or last section of the schizont, but elsewhere in the middle the cytoplasm is undivided and nuclear division may still be proceeding. As it is usually only at one end that this occurs and there is no evidence of it at the periphery of the section of maximum diameter in the few schizonts seen displaying this phenomenon it must be assumed that merozoite formation commences in one spot at the extremity and progresses more or less slowly throughout the schizont.

The merozoites themselves are very large, 1·8μ in diameter, and contain plainly delineated cytoplasm and nucleus which is easily demonstrable by Feulgen staining as well as by Giemsa stain.

The length of the pre-e cycle is 9 days and an e-e schizogony is presumed from the fact of the relapses reported by Garnham and his co-workers.

This completes the study of those parasites which are known to display the *cynomolgi* type of e-e schizogony. It can be confidently assumed that a similar pattern of e-e development is displayed by *P. gonderi*, *P. simium*, *P. pitheci*, *P. schwetzi* and *P. hylobati*, the tertian parasites of apes, by *P. brazilianum*, a quartan parasite of apes, and by *P. malariae*, the quartan parasite of man and apes. It is probable that *P. girardi*, the tertian parasite of lemurs, displays this pattern also.

P. falciparum Welch, 1897

Because of its relative susceptibility to radical cure by schizontocides and its resistance to prophylaxis by similar drugs it was considered that *P. falciparum* probably displayed only a pre-e generation, and that the tissue phase did not persist after the establishment of parasitaemia (Davey, 1946; Fairley, 1946, 1947; Huff, 1947). This view has been accepted by Shortt *et al.* (1949, 1951), Shortt (1951a) and Garnham (1951a). Garnham (1951a) has for this reason assigned *P. falciparum* to a grouping of its own in his study on the patterns of e-e schizogony.

The pre-e cycle of *P. falciparum* was described by Shortt, Fairley, Covell, *Shute and Garnham (1949, 1951) and confirmed by Jeffery*, Wolcott, Young and Williams (1951, 1952). Both these groups of workers introduced very large numbers of sporozoites into human volunteers and took liver pieces by biposy 5 days later. Shortt and his colleagues were immediately successful, using the methods already successful with *P. vivax* which included the biopsy of a relatively large piece of liver by open laparotomy operation. It is interesting to note that Jeffery and his colleagues failed to demonstrate the pre-e forms on 13 occasions when using less than 500 mosquitoes and taking liver snips by peritoneoscope. Only when

They are solid bodies of 100μ in diameter as observed by Edeson. Degenerating cysts resembling similar stages of *H. kochi* were also seen. They range from small abcesses to mere scar tissue. There is considerable reaction about the large cysts.

From the evidence available it seems definite that these processes represent the e-e stages of *P. knowlesi* since, if a mixed infection of *P. knowlesi* and a *Hepatocystis* sp. were involved, they should be distinguishable in the blood. Should *P. knowlesi* show a comparable speed of development to *P. falciparum* then such sizes of schizonts as have been described could be reached in 30 days. However, it can be taken for granted that no such forms occur in *P. falciparum* infections, as routine autopsies would have displayed them long before the present time. These cysts then are disturbing from a taxonomic point of view and it is difficult at present to see quite where to place them. The development is obviously not the *cynomolgi* or *falciparum* type and at the same time *P. knowlesi* is palpably not a member of the genus *Hepatocystis*. It must be assumed at present that a new pattern of e-e development is emerging from these studies. Such a new pattern may provide the link between the Haemoproteidae and the Plasmodiidae, having the tissue schizogony of the former and the blood schizogony of the latter. It is to be hoped that final elucidation will assist classification and not merely complicate it.

P. berghei Vincke and Lips, 1948

This parasite has been the subject of intense research in recent years. E-e forms have been described by Van den Berghe, Vincke and Chardome (1950) in histiocytes of the liver and bone marrow 43 hours after blood inoculation and during what they termed the "incubation period" Garnham (1951d) has demonstrated e-e parasites (*sensu stricto*) in normoblasts and other erythrocyte-forming cells in the bone marrow, but there is no proof that the parasites are able to reach maturity in these cells. I believe that this latter demonstration is an example of the great exuberance of multiplication and invasion displayed by this parasite, which inclines towards invasion of immature erythrocytes as a rule.

With regard to the Belgian workers' descriptions, Schneider and Schneider (1950) have shown conclusively that there is no incubation period after blood inoculation. A detailed and careful search of tissue taken under similar conditions by Dr. S. W. A. Kuper in this laboratory failed to reveal similar stages. I have failed to find such forms in the bone marrow although one young trophozoite was seen in what appeared to be a haemocytoblast. Similar failures have been reported by Galliard and Lapierre (1950), Fabiani, Vargues, Fulchiron, Grellet and Verain (1951), Mudrow-Reichenow (1951) and Baldi (1952).

The duration of the pre-e cycle after sporozoite inoculation has been established at 48 hours by Vincke and Peeters (1953) when the blood becomes infective. Such a short cycle is more typical of an avian type of development than of a

The 5-day-old form (see fig. 19) displays exuberant growth and assumes the more fantastic outlines, typical of *P. falciparum*. The parasite averages 50μ in diameter but a number of lobose arms are produced which make average measurements difficult. The cytoplasm shows meroblastic formation and there is some arrangement of the small nuclei about the meroblasts. Because of the formation of lobose protrusions, in certain sections the parasite may appear as two separate masses. The number of nuclei is probably about 10,000–20,000.

The 6-day-old form is mature and fantastic in shape. It is about 60μ in diameter with many indentations and protrusions. The number of merozoites is about 40,000 and they retain the patterning of the former meroblasts, thus simulating a true cytomere formation. The merozoites are small, measuring about 0·7μ *in diameter. At no time are vacuoles a feature of this parasite. The 20μ form* found on the 3rd day precludes any possibility of a prior pre-e generation.

The duration of the pre-e generation is 5–6 days (Fairley, 1947; Shortt *et al.*, 1951). De Sanctis Monaldi (1935) has recorded a blood parasite 4½ days after infection and Raffaele (1946) and De Sanctis Monaldi and Raffaele (1953) have stressed that the incubation period of *P. falciparum* may be less than 5 days, but here as with *P. vivax* they refer to the Italian strain of the parasite. Shute also claims to have seen blood parasites at times of less than 5 days after sporozoite inoculation. The work of Fairley and Shortt *et al.* however, is absolutely conclusive and it remains only possible that strain differences occur, as the different observers used geographically widely differing strains. Raffaele used an Italian strain, Shute a West African strain, Fairley a New Guinea strain and Shortt *et al.* a Rumanian strain. A similar type of development is probably displayed by *P. reichenowi*.

P. knowlesi Sinton and Mulligan, 1932

It had been hoped to provide some description of the pre-e cycle of this parasite, as a result of work initiated by Professor Garnham just prior to the time of writing. Unfortunately, the parasite has proved more refractory to mosquito transfer than was expected and further study has been deferred.

Edeson (1953) has described "presumed exo-erythrocytic schizonts of *Plasmodium knowlesi* in the liver of a Malayan monkey". The apparently mature *forms are large cysts, 1·5 mm. in diameter as seen in sections* of liver from 4 monkeys (*M. irus*) found to be naturally infected with *P. knowlesi*. These cysts resemble the merocysts of *H. kochi*, and even more closely those of *H. tassali* as described by Ray (1949). There are a number of finger-like processes radiating out from a central vacuole and these projections appear to contain the parasite matrix, including merozoites. The vacuole contains a basophilic substance, probably the colloid material found in *H. kochi* (Garnham and Pick, 1952) and some parasitic material can be seen on the periphery. The immature schizonts resemble the 15-day-old *P. cynomolgi* forms or the young schizonts of *H. kochi*.

forms of *P. cynomolgi*, it must be assumed that they have some effect upon pre-e forms of mammalian malaria. That this effect is commingled with some residual effect upon erythrocytic progeny, at least in the case of pyrimethamine, must also be accepted. The present explanations advanced for these various effects are far from satisfactory and show too great a tendency to place the blame for all inexplicable events on to hypothetical and special tissue-inhabiting stages. It must be stressed that the e-e cycle is not the *deus ex machina* explanation of all the troubles which beset the workers in the field of antimalarials. The e-e cycle is a biological phenomenon microscopically visible and, as such, must be studied for basic information.

Setting aside for the moment the effect of residual active metabolites of proguanil or pyrimethamine upon erythrocytic forms, the actual effect of the two drugs upon *P. vivax* and *P. cynomolgi* sporozoite-induced infections is difficult to explain. It can be imagined that one of three sequences of events occurs. (*a*) All pre-e forms are eradicated, as is supposed to occur in *P. falciparum* infections. (*b*) The drugs eradicate most but not all of the normal pre-e forms. (*c*) The drugs cause a temporary cessation of growth in some forms or are inactive against some forms naturally slowly growing or arrested while the remainder are eradicated.

Obviously the first of these does not occur. Should the second suggestion be true, it is difficult to understand why a delay in parasitaemia should be so long. If merozoites on the 8th and 9th days invade the blood in numbers sufficient to cause an infection, then the delay would not be longer than the 5–10 days necessary for multiplication to patent levels except in strains where long-term latency is common. That is to say, the blood infection would be normal, as for a low sporozoite level of infection. The third suggestion is the only possible alternative. There are in fact two theories involved here : (i) that these drugs cause a general lengthening, by some slowing down or temporary cessation of growth, of the pre-e cycle ; (ii) that forms exist naturally which are more slowly growing and resistant to the drugs. Hawking and Thurston (1952) have shown that sub-effective doses of proguanil cause an excessive vacuolation and some hypertrophy of the parasite. This does not fit the notion of some retardation or cessation of growth due to drug action. One is left with the proposal that forms occur, not seen by Hawking and Thurston, which while retarded are not affected by the drugs and which fully retain their capacity to produce merozoites at a time later than the majority of pre-e forms.

Such forms could be encysted, arrested or retarded, and as has been said, the two former possibilities are rejected. There remains the retarded forms already described in an earlier section. If these forms are truly growth-retarded but otherwise normal and growing schizonts, as is believed to be the case, it may be assumed that the activity of their nuclear division is at a minimum. Such forms would then be resistant to proguanil or pyrimethamine drugs, which are schizontostatic rather than schizontocidal and which act largely upon the late nuclear

tion with and under the direction of Professor Shortt the present author set up an experiment to test the efficacy of proguanil under similar conditions. It was not possible to demonstrate pre-e forms, but a prepatency of 31 days was recorded.

There is much indirect evidence of drug action upon e-e forms of mammalian malaria. Information on the presumed action of various drugs upon e-e forms can be obtained from observations concerning prophylaxis, radical cure, delayed parasitaemia and reduced relapse rate. This information is tabulated in table 2 (see page 115).

Useful reviews have been published by Coatney and Cooper (1948), Cooper (1949), Fairley (1949, 1952) and Mudrow-Reichenow (1950, 1952).

G. M. Findlay's volume of *Recent Advances in Chemotherapy* (1951) dealing with antimalarials has proved invaluable in the study of this aspect.

It remains doubtful whether proseptasine is a causal prophylactic in *P. falciparum* infections, as Sinton's experiments with it (Sinton *et al.*, 1939) give no indication of whether it is the pre-e forms or the early erythrocytic forms which are attacked. Ardias (1948) stated that sulphone therapy following Ascoli's adrenaline treatment lowered the relapse rate in *P. vivax* infections, but this has no significance *vis-à-vis* the e-e forms, as the treatment is based upon the Bignami theory of relapse and attack on erythrocytic parasites driven from the spleen.

From the table it can be seen that only the 8-aminoquinolines have the capacity of completely eradicating the pre-e and e-e forms of mammalian malaria. However, to effect this with certainty, with all of the groups except primaquine, toxic or near toxic dosages are necessary. Primaquine in well tolerated doses has been successful in eliminating relapses from the Korean strains of *P. vivax* (Garrison *et al.*, 1953; Alving *et al.*, 1953).

The biguanide group and pyrimethamine are causally prophylactic in sporozoite-induced *P. falciparum* infections, indicating a complete eradication of the pre-e forms. This is, however, only a presumption, as Coatney *et al.* (1953) have shown that pyrimethamine is active after a single dose for up to 17 days and its action may be one of suppression upon the early subpatent blood infection. Spinks (1946) showed that proguanil is largely excreted in the first few days, however, it may be that the active triazine metabolite (Carrington *et al.*, 1951) is retained for periods comparable to those of pyrimethamine. The drugs are nothing like as successful in dealing with sporozoite-induced infections of *P. vivax* and *P. cynomolgi*. Both proguanil and pyrimethamine cause some delay in the primary parasitaemia in both infections. Pyrimethamine causes a delay of 20 to hundreds of days while proguanil causes shorter delays of about 20 days. These effects appear to be more or less independent of sporozoite dosage. Neither has any effect upon the relapse rate following the primary parasitaemia and therefore presumably no effect upon the e-e forms.

As both drugs have some effect upon e-e forms of avian malaria and as Hawking and Thurston have shown that proguanil apparently eradicates some pre-e

largely insensitivity to proguanil and pyrimethamine, of a strain of mammalian malaria, by treatment of the blood infection, confers resistance upon the e-e forms as well. Resistance is carried through both sporogony and pre-e schizogony (Schmidt *et al.*, 1949; Cooper *et al.*, 1950; Hernandez *et al.*, 1953). Also, proguanil-resistant strains of *P. falciparum* are resistant to prophylactic doses of drug (Walker and Reid, 1953). This faculty, however, appears to be carried by the gametocytes and cannot be acquired directly by the e-e cycle during regimens so far tested as, if blood forms are made resistant in an animal and then eliminated, the new blood forms from a true relapse are sensitive (Schmidt, 1953).

Biochemistry

There is almost no published information upon the physiology and biochemistry of the e-e forms of mammalian plasmodia. Much of the information relating to e-e forms of avian malarias is probably relevant, particularly where drug action is similar and conclusions have been based on chemotherapeutic considerations. Thus it may be assumed that the folinic acid complex, with its precursors and reactants, is active in the mammalian forms, as proguanil and sulphadiazine are active. This complex, instrumental in the synthesis of nucleosides and nucleic acid, is necessary for the supply of deoxyribonucleic acid (DNA) which, as will be shown, is present in the final division stages of pre-e forms. For the mammalian liver forms there are, at present, only three sources of information concerning their biochemistry. Firstly, knowledge of the special physiology of the liver and the biochemical aspects of its various functions may possibly give some indication why this site is so exclusively favoured by the parasite and what compounds the liver possesses to allow the rapid growth of the parasite. Secondly, histochemical treatment can supply a certain limited number of facts. Thirdly, as has been seen, chemotherapeutic and drug interference allows some indirect conclusions.

Telcharov and Todorowa (1950) suggested that the presence of abundant glycogen in the liver was the reason for the selection of the liver parenchyma as the site of development of the parasite. They further suggested that the glycogen provided the carbohydrate source for the active growth of the schizont. In early 1952 Dr. Williamson and I suggested to Professor Shortt that the uptake of glycogen into the parasite might well be determined qualitatively. Investigation of various means of glycogen depletion of liver cells led to the choice of the drug phlorhizin which causes the kidney to extract rapidly and eliminate blood sugar, thus causing a constant flow to exhaustion of carbohydrate from the liver. This method was superior to the use of insulin which causes only a temporary and transitory loss of liver cell glycogen (Soskin and Levine, 1946). It was decided then, in conjunction with Dr. Williamson, to investigate this method of liver glycogen depletion and to determine the effect of such a depletion on the pre-e cycle of *P. cynomolgi*. The results have been briefly demonstrated (Bray and

divisions accompanying schizogony. There may be a further division in drug activity into little action against young schizonts and maximum action against old schizonts. It is known that proguanil and pyrimethamine act at the time of maximum nucleic acid exchange and when deoxyribonucleic acid is demonstrable in the parasite nucleus. This condition obtains only in the older schizonts if we are to believe the results of the Feulgen reaction (see page 125). Thus the retarded forms may possess not only the advantage of little nuclear activity but also a type of activity which is not susceptible to attack at this stage in any case. Such forms, retarded to the extent of drug resistance, would take 20–25 days to reach maturity and produce their relatively low total of blood-invasive merozoites. Thus, the primary parasitaemia initiated at this time would not become patent until about 30 days after infection. It is to be stressed that this explanation does not suffice to explain the long-term delays brought about in some cases by pyrimethamine in South-West Pacific strains of *P. vivax*, where some other effect is obviously acting.

A further unexplained fact is the differential action of proguanil or pyrimethamine on *P. falciparum* infections and *P. vivax* infections when the drugs are administered as prophylactics. The action of pyrimethamine can be wholly explained by the residual action of persisting drug in the plasma on early erythrocytic infections in the case of *P. falciparum* where, as no e-e infection exists, the action is radical. In this case the claim that pyrimethamine is active against pre-e forms of *P. falciparum* is baseless on the present information, though no doubt such activity exists. However, in the case of proguanil it appears as if some action against the pre-e forms occurs, as drug plasma levels after single early dosage are accepted as being too low to affect the erythrocytic phase when it occurs, although the author reserves opinion until further pharmacological investigation has been made. When proguanil is considered it is not enough to state that *P. falciparum* undergoes only one pre-e generation and that this is eradicated, whereas *P. vivax* displays a persisting tissue phase. If the action upon the forms themselves were similar, then no clinical attack could be expected in *P. vivax* infections whether sooner or later. It must be assumed that there is some difference in the mode of growth between the pre-e forms of *P. vivax* and those of *P. falciparum*. Such a difference might be a biochemically fundamental factor in closely similar cycles or a different mode of growth resulting in dissimilar schizogony. The present author feels that there is sufficient evidence to postulate a different mode of growth in the pre-e generation between the two parasites and the plasmodia closely allied to them. *P. falciparum* reaches a greater size than *P. vivax* in about two-thirds of the time and it is postulated that such a rate of growth precludes the existence of retarded forms with a sufficiently slow growth and nuclear division to resist the action of proguanil. In *P. vivax* infections, it is presumed that progunail-resistant retarded forms do occur.

It would seem on general evidence that acquired or natural drug resistance,

Day + 7	Animal anaesthetized. Liver frozen *in situ*. Animal killed and frozen liver pieces taken for glycogen analysis. Other unfrozen and frozen pieces were fixed in Carnoy's fluid, hot and cold formol-alcohol for later embedding and sectioning.
M124	Normal diet throughout.
Day 0	Infected as above.
Day + 7	Killed and liver taken as above.
M125	Excessive carbohydrate diet throughout. Animal fed 10% solution glucose in milk, carrots, potatoes and large amounts of glucose for which the monkey displayed considerable greed.
Day 0	Infected as above.
Day + 7	Killed and liver taken as above.

All mosquitoes were obtained from a single batch of 700 *A. maculipennis atroparvus* infected with gametocytes of *P. cynomolgi* as a batch. Liver samples were taken after freezing by dry ice *in situ* before death. Glycogen analysis was made, by means of the sugar reagent of Somogyi (1945). Sections of liver were stained with Giemsa stain differentiated in colophony or acetic acid, with Best's carmine, Bauer Feulgen, Feulgen-light green, Gomori's silver methenamine and Danielli's modification of Gomori's method for demonstration of alkaline phosphatase.

In this experiment, the results of which are set out in table 3 (page 122), it was shown that glycogen depletion increased the schizont content of the liver fivefold. This was a most surprising result. The 7-day-old pre-e schizonts in all monkeys were typical and no signs of degeneration, no alterations in size or number of vacuoles, were observed in the treated monkey. Schizont diameter measurements averaged 36μ in each of the three monkeys and vacuole numbers averaged 2·3, 2·2 and 2·3. Stain reactions were similar. The only observed morphological difference observed was the predominance of bacilliform nuclei in M124, and this was considered to be of no significance. Thus, the effect recorded was purely one of increased invasion of liver parenchyma by sporozoites.

Two explanations of this effect are possible. Firstly, it might be thought that phlorhizin treatment depressed endothelial macrophage activity and consequently more sporozoites were able to reach the liver. This seems unlikely as no comparable records exist in the literature on phlorhizin and treatment was begun only the day before sporozoite inoculation. Furthermore, there was no difference in intensity of *P. berghei* infections between treated and control mice. Secondly, it can be assumed that glycogen acts as a barrier to parasitic invasion as it is known to do in some virus diseases of the liver. On glycogen depletion this barrier is removed and greater numbers of parasites are successful in penetrating the parenchyma cells.

Some observers have noted the effect of sporozoan diseases upon liver glycogen levels (Fulton, 1939; Pratt, 1941) but no data are available on the effect of various

Williamson, 1953) and the full details will be given here. It is with the permission of Dr. Williamson that this work is recorded here.

It was first necessary to determine the toxicity of phlorhizin when dissolved in propylene glycol and to investigate its effects upon malaria parasites. Following this, the drug was tested for its efficiency as a glycogen-depleting agent in monkeys and a satisfactory method of determining liver glycogen had to be established.

It was found that the solvent—propylene glycol— was toxic to mice at levels of 0·2 ml. This was reduced to 0·05 ml. The maximum tolerated dose (chronic toxicity) of phlorhizin was found to be in the region of 250 mgm. in 0·5 ml. propylene glycol per kgm. body weight. A dosage of 100 mgm. in 0·5 ml. per kgm. body weight was found to be well tolerated by both starved and normal monkeys over eight days of daily intramuscular injections. Phlorhizin was shown to have no effect on the course of *P. berghei* infections in white mice.

Liver samples from mice, rats and monkeys were tested for liver glycogen content after single and multiple dosages of phlorhizin in order to test both the efficacy of the drug and to standardize the methods of glycogen estimation. The methods of glycogen estimation used were those of Good, Kramer and Somogyi (1933) using the colorometric method of Nelson (1944) and the sugar reagents of Somogyi (1945) or his later modification (Somogyi, 1952). It was found that the above regimen of phlorhizin treatment over 8 days virtually eliminated liver glycogen in starved monkeys.

The findings of Morrione and Mamelok (1952) were also confirmed with regard to the speed and extent of glycogenolysis in the liver on death or excision. Results showed that despite immediate immersion in boiling potash, liver pieces removed normally displayed a sharp and considerable loss of liver glycogen when measured chemically. Only when the whole liver was frozen *in situ* with the animal under deep anaesthesia could reproducible results be obtained from all lobes of the liver. The pieces were then literally chipped off and placed directly into boiling potash solution. By this method it was possible to establish that phlorhizin combined with starvation was more effective in depressing liver glycogen than starvation alone.

With this information an experiment was performed with 3 *M. mulatta* on the following regimens :

M123

Day – 1	All food removed. Animal offered water only. Inoculated intramuscularly with 100 mgm. phlorhizin in 0·5 ml. propylene glycol per kgm. body weight.
Day 0	Starved. Treated with phlorhizin. Intravenously infected with 100 glands of *A. maculipennis atroparvus* heavily infected with *P. cynomolgi*.
Day + 1 to + 6	Starved. Phlorhizin given daily.

attack. *Thirdly, this effect may have some influence, as yet incalculable, in* endemic areas of malaria with associated high degrees of malnutrition where a high threshold of sporozoite attack is probably necessary to overcome immune responses. Thus a state of malnutrition causing a low glycogen level may also lower the threshold number of sporozoites necessary to establish an infection.

Such conclusions are to some degree borne out by the case of monkey M125 *which is not recorded in table 3 above.* This monkey ingested enormous amounts of carbohydrates. Unfortunately, neither blood sugar nor urine sugar contents were estimated. The liver glycogen content of the M125 after 8 days of high carbohydrate intake was found to be only 1·71% where a figure approaching 12–15% had been expected. The only satisfactory explanation seemed to be that the monkey was diabetic. The schizont content of the liver proved to *13,100* per cm.^{3}, which being a twofold increase over the control, is in accordance with a low glycogen content at all times during the experiment. From this it might be assumed that a diabetic could expect double the normal number of pre-e schizonts to develop in his liver on mosquito infection.

Some rough calculations were made during this experiment in an attempt to estimate the number of sporozoites inoculated and the number of pre-e schizonts to be subsequently found in the whole liver. Should the estimated number of schizonts significantly exceed the estimated number of sporozoites introduced, this would argue an intervening generation. It was calculated that about 3×10^6 free sporozoites, and at least that number entangled in gland debris, were inoculated. The calculated number of schizonts in each of the three livers was considerably less and thus no conclusions could be drawn. The figures are given in table 4, below.

TABLE 4

No. of free sporozoites inoculated	*M123* 3×10^6	*M124* $3{\cdot}5 \times 10^6$	*M125* $2{\cdot}5 \times 10^6$
No. of schizonts in the whole liver	*$1{\cdot}5 \times 10^6$*	*4×10^5*	*$9{\cdot}8 \times 10^5$*
Ratio of free sporozoites/schizonts	2	8·5	2·5

It is stressed that the figures for the number of sporozoites inoculated per schizont produced are probably much too low as at least double the number of sporozoites shown were probably inoculated. It is also worthy of mention that, in all experiments carried out with either *P. cynomolgi* or *P. inui* in which sporozoite counts have been made, schizont numbers have shown a reasonable correlation with sporozoite numbers irrespective of the number of mosquitoes used.

An interesting theoretical consideration arises from these results. Raffaele (1952) has expressed doubts as to the ability of the sporozoite successfully to pass

TABLE 3

Monkey	Infection *P. cynomolgi*	Treatment	% glycogen in liver	Best's carmine reaction	Pre-e schizonts
M82	none	water only	1·8 ± 0·05	—	
M93	none	water only phlorhizin i/m	0·35 ± 0·01	—	
M91	none	Normal diet	8·2 ± 0·2	+	
M123	100 glands i/v	water only phlorhizin i/m	>0·10	—	29,100/cm.^{3}
M124	100 glands i/v	Normal diet	8·7 ± 0·2	+	5,600/cm.^{3}

glycogen levels upon protozoa. It is known that the loss of vaginal glycogen may precipitate proliferation of *Trichomonas vaginalis*, but this parasite is neither intracellular nor apparently directly affected by glycogen levels as the effect is more likely bound up with *p*H changes due to glycogen loss and other concomitant alterations in physiological activity. However, the latter explanation is more satisfactory and has been adopted as a working hypothesis.

The other main fact which emerged from this experiment was that the pre-erythrocytic schizont of *P. cynomolgi* does not utilize stored liver glycogen as a source of carbohydrate. It seems probable that, as in the erythrocytic stages, only monosaccharides are metabolized, and the source of these is the blood sugar.

The information available upon malaria associated with diabetes has recently been reviewed by Born (1952). Born found that there was some connection between the two diseases, and that the onset of diabetes frequently coincided with a malarial attack. Glycosuria is known to increase with antimalarial therapy. None of this, however, is very relevant. From the results given above it might be taken that a diabetic will become more heavily infected than a normal person when inoculated with a comparable number of sporozoites. This has no clinical significance as the malarial attack would be no different. It may have some significance in three ways. Firstly, the relapses may be more frequent in a diabetic, owing to a greater number of e-e schizonts thus producing threshold numbers of blood-invading merozoites at more frequent intervals. Secondly, if the sporozoites inoculated are for any reason enfeebled, a diabetic person may suffer a normal primary attack when a normal person would have undergone a delayed primary

6. The above haematoxylins counterstained with Orange G, eosin-azur II, methyl blue and Giemsa.
7. Mann's stain. Carnoy, Helley and formol-saline fixation.
8. Gram's stain. Carnoy fixation.
9. *Phloxine, toluidine blue, Orange G staining. Acetic acid and colophony differentiation.* Carnoy fixation.
10. Feulgen stain and methyl blue or light green counterstain. Carnoy and formol-alcohol fixation.
11. Toluidine blue with or without prior treatment with ribonuclease and with or without alcoholic differentiation. Carnoy and formol-alcohol fixation.
12. Best's carmine. Carnoy and formol-alcohol fixation.
13. Bauer Feulgen. Carnoy and formol-alcohol fixation.
14. Gomori's silver methenamine. Carnoy and formol-alcohol fixation.
15. Danielli's modification of Gomori's stain for alkaline phosphatase. Carnoy and formol-alcohol fixation.
16. *Phloxine-tartrazine stain. Carnoy fixation.*
17. Alizarin Red S, phosphomolybdic acid. Carnoy fixation.
18. Leuco-fuchsin, methyl green. Carnoy fixation.

Methods 1–9 were adopted in order to estimate the optimum routine staining method. It was found that no method was as successful as the routine Giemsa colophonium stain when this was used on tissue fixed in Carnoy. This was not only true for pre-e forms but also for blood forms in capillaries. The pre-e stages of *P. cynomolgi* are Gram-negative. Method 10, Feulgen staining, has proved most satisfactory for the staining of final division nuclei of *P. inui*, *P. ovale* and *N. medusiformis*, as has been shown by Garnham (Garnham and Heisch, 1953; Bray and Garnham, 1953b; Garnham *et al.*, 1955). Results with pre-e stages of *P. cynomolgi at similar stages have been disappointing. 7- and 8-day-old forms are* Feulgen negative, and the 10-day-old bursting schizont is only faintly Feulgen positive when compared with similar stages of *P. inui* or *P. ovale*. It will be remembered that whereas *P. inui* shows definite nuclear patterning and, as will be seen, *N. medusiformis* displays mitotic-like figures, *P. cynomolgi* shows virtually none of this type of activity. Thus it would appear that DNA is not present in any considerable amounts in the early forms of *P. cynomolgi* and, even at the final stage, is only weakly demonstrable. On the other hand, DNA is reasonably heavily deposited in nuclei in the final stages of *P. inui* and *P. ovale*. It is interesting to note that the mature forms of *P. falciparum are strongly* Feulgen positive, indicating a heavy deposition of DNA in the merozoites (Young, 1953). It is known that proguanil inhibits the DNA producing process (Lewert, 1952b) and so it might be assumed that the difference in DNA content of nuclei of *P. falciparum* and *P. cynomolgi* might be a contributory factor in the different action of proguanil upon the pre-e forms of the two parasites.

The above results should not be interpreted as an indication that little biochemical change *occurs in the nuclei of P. cynomolgi* pre-e forms. I am convinced that profound and continual physiological processes are taking place at all times in the nuclei. It has been observed while studying tissue cultures that, when stained

the R-E system and arrive at a parenchyma cell. This is not the present author's understanding of the action of the R-E system under such conditions. In the absence of any specific humoral activity, the cellular action will take some time to clear all circulating sporozoites in the absence of specific activation. This time is presumably the hour during which the blood is known to be infective. During this time some sporozoites may pass through the liver several times and the majority of the sporozoites should have an opportunity to enter the organ once. It is known that blood can traverse the body in 25 seconds and although blood flow through the liver is sluggish, the amount of blood in the liver at any one time is very large. Thus it seems that the fate of a sporozoite is as much bound up with its ability to penetrate a parenchyma cell of the liver as with the hazards of reaching such a cell. Should Raffaele be correct in his assumption then there should be no difference in parasitic invasion between normal and glycogen-depleted monkeys when it is remembered that Raffaele does not argue that pre-e schizonts do not occur in the liver but only that they occur in endothelial, not epithelial, cells.

On the other hand it is here postulated that the glycogen stores of a parenchyma cell play a considerable part in the establishment of a pre-e infection. *Should the glycogen barrier refuse entry of a sporozoite on its first passage through* the liver, the hazards of endothelial engulfment of the sporozoite must be once more undergone before a second chance is offered, and the lymphoid macrophage system may claim it within the hour. If, however, the glycogen barrier is removed, the potentially less successful sporozoites may gain entry into a parenchyma cell, effecting a higher rate of infection than normal. The ratio of inoculated sporozoites to resultant pre-e schizonts would appear to indicate that a sporozoite may be offered more than one chance of passing through the liver and invading polygonal cells.

It would be interesting to speculate upon the statement of Garnham (1951a) to the effect that nothing can make *P. elongatum* develop in a parenchyma cell of the liver, but further experimentation is needed.*

The next source of biochemical information is the result of histochemical investigation of pre-e forms of mammalian plasmodia. Of the various results set out below those which refer to *P. cynomolgi* are the work of the author. The following stains and treatments have been tested with pre-e schizonts of *P. cynomolgi* 7, 8, 10 and 15 days old, fixed in Carnoy, formol-alcohol, formol-saline or Helley's fluid. Carnoy fixation has been used as routine.

1. Giemsa stain, colophony or acetic acid differentiation. All fixatives.
2. Heidenhain's iron haematoxylin. Carnoy fixation.
3. Delafield's haematoxylin. Carnoy, Helley and formol-saline fixation.
4. Erlich haematoxylin and eosin. All fixatives.
5. Mallory's iron haematoxylin. Carnoy, Helley and formol-saline fixation.

* Very recent work by Dubin in which he has shown that *P. gallinaceum* will grow in epithelial cells *in vitro*.

Carnoy's fluid for 5 minutes, embedded, sectioned and stained with Glemsa-colophonium.

EXPERIMENT II

The details are essentially the same as previously, with the following modifications : fowl plasma replaced human plasma, clot liquefaction was sucked off when necessary and transplantation was carried out daily, sporozoites were obtained without asepsis and suspended in Tyrode fluid containing 100 units penicillin and 100 μgm. streptomycin per ml.

EXPERIMENT III

Coverslip cultures of monkey liver and spleen were prepared by the method of Hawking (1944). Antibiotics were used as above and sporozoites of *P. cynomolgi* in very large numbers were introduced into the fluid phase of Tyrode fluid and monkey serum. Tissues were harvested at intervals of 6 hours during 36 hours, and treated as above.

EXPERIMENT IV

Roller tube cultures of HeLa cells (cervical carcinoma cells) growing on glass were inoculated with sporozoites of *P. vivax*. The sheets of fixed and stained tissue were removed on celloidin films or on wax and floated on to glass slides.

Results, Discussion and Conclusions

In Experiment I, no evidence of pre-e forms was found and no evidence of growth or even life could be seen in the parenchyma cells of the explants. Even 6 hours after biopsy the explants appeared to be dead. Transplants harvested after 2 days and 6 days both showed some signs of life. A concentric ring of live tissue could be seen halfway between the periphery and the centre. The live tissue was exclusively endothelial as far as could be determined. All the remainder of the tissues were dead and showed extensive pyknosis, karyolysis and necrosis. There was excessive liquefaction of the soft human plasma clot and this was thought to have poisoned the normally healthy periphery of an explant.

In Experiment II, with the hard fowl plasma clot, similar but slightly better results were obtained with regard to the liver growth. The concentric ring of live tissue was more extensive and lasted up to 12 days. However, it was still endothelial and no parasites were demonstrated. Professor Adler has informed the writer of very similar results obtained in comparable tissue culture experiments carried out in his laboratory. It was agreed that even if some liver tissue could be kept alive, the rapid death of peripheral cells would disallow parasitic entry and growth.

In Experiment III, although numerous sporozoites were seen to lie free, none

mentioned, a certain requirement is involved for cobalt, the central atom of the vitamin B_{12} chelate. Whether or not the cobalt is to any extent retained in the parasite is not necessarily important, but that it is essential at some stages is certain. This has been mentioned not because of its importance in chemotherapy but because of its possible use as a radio-active tool in tracer experimentation. Radio-active cobalt introduced in the vertebrate would probably collect in the nuclear-labile parasite to a far greater degree than in the surrounding nuclear-stable liver tissue. Such a technique would, unfortunately, be of little use in distinguishing very young pre-e forms, as nuclear division at this stage is not active.

Tissue Culture

The relative difficulty of producing mammalian e-e forms at will for the myriad experiments which a worker would like to perform with them made attempts at the tissue cultivation of the schizonts a *sine qua non* for an investigator.

Dubin (1947, 1948) attempted to cultivate *in vitro* the e-e forms of *P. vivax* in bone marrow taken from induced *P. vivax* infections in patients with general paresis. Dubin, Laird and Drinnon (1950) attempted to infect liver explants with sporozoites of *P. vivax*. None of the attempts was successful. Dubin and his colleagues found that the liver growth was purely fibroblastic and thus the parenchyma-inhabiting pre-e forms could not be expected to grow.

The present author has made four essays at this most difficult of tasks and the experimental details will be recorded briefly here.

Materials and Methods

In the first two attempts the techniques used were those of Fell (1929), designed to maintain differentiated tissue *in vitro*. Some controlled growth is obtained by this method, but as it would have been sufficient to keep liver tissue alive for one or two weeks, all techniques were directed to that end.

Experiment I:

18 clots of human plasma and chick embryo extract in Tyrode solution were set up in watch glasses and placed in moist chambers. Monkey liver was taken by needle biopsy, kindly performed by Professor Woodruff. The liver piece was cut into 18 explants of approximately 1 mm. diameter and placed on the clots. These were incubated at 37·5°C. Glands of 18 *A. maculipennis atroparvus* heavily infected with sporozoites of *P. cynomolgi* were removed aseptically without severence of the alimentary tract, after the method of Gordon and Hill (1946). The sporozoites were suspended in Tyrode solution and one drop of this solution containing the glands of one mosquito was placed on each explant and incubated for 6 hours. The glands and medium were then sucked off. The explants were then incubated for periods of up to 12 days. Tissue pieces were transplanted to new clots every two days and pieces were harvested daily. The pieces were fixed in

Carnoy's fluid for 5 minutes, embedded, sectioned and stained with Glemsa-colophonium.

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In Experiment III, although numerous sporozoites were seen to lie free, none

were demonstrated in cells of either the spleen or the liver at any time. Spleen was seen to be actively growing after 36 hours, but once again the liver seemed to be dead. As there has been no record of successful growth or maintenance of adult mammalian liver parenchyma *in vitro* it was thought futile to carry on this line of research.

Experiment IV had been designed so as to use an actively growing culture of human foetal liver cells.* Unfortunately this system was lost a few days before sporozoites were to be harvested and so the HeLa cell system was substituted. No growth of the parasite was seen. I have to thank Dr. Kingsley Sanders for his co-operation both in supplying the HeLa cell culture and his continuing efforts to grow foetal liver on my behalf.

One interesting point arose from this work. From the results it seems that parenchyma cells die within 6 hours after excision when transplanted into cultures. It is already known that glycogenolysis and perhaps other changes occur within the parenchyma cell instantaneously on death or excision. Huff (1953c) has remarked that one of the essential pre-conditions for the acceptance of the existence of mammalian pre-e forms in liver tissue is the infectivity of that tissue at a time of prepatency in the blood. It is my belief that although this is possible, this pre-condition will not be established except by extensive and expensive research or by pure chance. Mulligan *et al.* (1949), Shortt and Garnham (1948c) and Hawking *et al.* (1948a) have all failed to transmit *P. cynomolgi* by inoculation of macerated liver on the 7th and 8th days prior to the onset of the donor's parasitaemia. Further, and what makes one somewhat impatient with this pre-condition, Huff (1942) has failed to transmit *Leucocytozoön* by inoculation of spleen emulsions, as have other workers.

The following facts concerning the liver and the pre-e infection of *P. cynomolgi* must be borne in mind :

1. Liver parenchyma dies on excision and transplantation within 6 hours. Certain changes occur instantaneously in the parenchyma cell which might possibly be fatal to the parasite.
2. The blood becomes infective on the rupture of the first pre-e schizont but a large proportion of pre-e schizonts do not reach maturity until 12–48 hours after this time.
3. The pre-e generation lasts 8 days and the last maturation changes and final merozoite formation within the pre-e schizont are swift, perhaps confined to 12 hours, and require considerable metabolic turnover.
4. Similar techniques of inoculation have failed to transmit the accepted schizogony stages of *Hepatocystis* and *Leucocytozoön*.

* It is not known, of course, whether the pre-e cycle of mammalian plasmodia will grow in foetal parenchyma even *in vivo*. This raises a point which should be resolved some time in the future as it may add yet one more factor to the growing list of possible causes for the curiously low incidence of malaria in infants aged 0–3 months in hyperendemic areas.

It is in the light of these facts that Huff's essential pre-condition must be examined. *In avian malaria the cycle of development is 36 hours and occurs in* endothelial cells which continue to live for periods much longer than 36 hours on transplantation either *in vivo* or *in vitro*. Infectivity of avian pre-e forms is thus easily understood. In mammalian malaria it would be necessary to choose a time in the donor's pre-e infection when schizonts are nearing maturity. At a time of say 180 hours after *P. cynomolgi* sporozoite infection, when it is fairly certain that no pre-e schizonts have ruptured, a few schizonts might be inoculated when undergoing their last stages of schizogony. On excision the liver cells die within 6 hours, perhaps within one hour, and will not then support life. This is the time when profound metabolic changes are occurring in the parasite. At later times, when some schizonts may have reached full merozoite formation, the blood may be already infected, owing to the bursting of one schizont with its thousands of merozoites. Thus, for success in such a venture, a heavy concentration of pre-e forms would be necessary and the time of inoculation either carefully calculated or brilliantly inspired , all this to be repeated with many monkeys. What might be more possible would be the inoculation of infected liver 8½ or 9 days after infection and the demonstration of second-generation e-e forms in the recipient while the state of the blood infection was ignored. This would not, of course, satisfy Huff's criterion.

(c) RELAPSES, IMMUNOLOGY, PATHOGENICITY

Relapses

In this section it is not intended to argue from observed biological phenomena to the syndromes of a clinical relapse, but rather to infer biological data and states of parasitism from clinical and chemotherapeutic data and correlate such inferences with the scanty known biological facts. Thus it will suffice to say that one and only one biological fact is available. Persisting e-e forms of *P. cynomolgi* have been described 102 and 105 days after sporozoite inoculation (Shortt and Garnham, 1948e; Shortt, Bray and Cooper, 1954).

Firstly it is desirable to examine the evidence put forward by various authors for a genesis of relapses other than the presence of e-e forms. Some Italian workers hold to the Bignami theory of the genesis of relapses which relates the reappearance of patent parasitaemia to a persisting sub-patent erythrocytic infection. Ascoli and Alessandro (1950, 1951) have pointed to the reduction of the relapse rate obtained with the Ascoli adrenaline-schizontocide therapy as a proof that some relapses are due to low-level schizogony in the blood of the viscera. They believe *that not only are the e-e forms not the "deus ex machina* explanation of relapses", but also that the e-e forms are but a transitory stage. They support their contentions with references to the persistance of pigment which has already been discussed in this memoir, and to the work of Corradetti and Verolini (1950, 1951)

discussed below. Ascoli and Alessandro believe that two generations of pre-e development exist, the first being that described by Coulston (1949) and the second the well known parenchyma cell generation. These are thoughtful papers, but there are certain essential omissions. No differentiation between relapse and recrudescence is made, nor is there any recognition of the differences existing between the sporozoite- and the blood-induced infections of *P. vivax*. No mention is made of the persisting e-e stages of *P. cynomolgi* found after a negative blood phase which surely puts to flight the fancy that e-e forms are merely transitory. Similar misunderstandings are evident in the work of Corradetti and Verolini (1950, 1951).

These authors studied the relapse phenomenon in blood-induced infections of *P. cynomolgi* in monkeys and *P. malariae* in man. They found that " relapses " occurred in both infections after a period of negative parasitaemia. They reported that the liver of the blood-infected monkeys contained no demonstrable e-e forms. Some of the " relapses " occurred only 2 weeks after the apparent disappearance of the blood phase. Several comments are called for in relation to this work. Firstly, as e-e forms do not occur in blood-induced infections of simian or human malaria it is not surprising that none was found. Secondly, although the blood was negative by microscopic examination in the intervals between relapses, it was not shown to be negative on inoculation, which is the essential criterion in these cases. As Fairley (1947) has shown in blood-induced *P. vivax* infections and Schmidt (1953) has shown in blood-induced *P. cynomolgi* infections that the blood is infective between parasitic recrudescences, I cannot accept this aspect of the reports of Corradetti and Verolini. Thirdly, the very short period between recrudescences of blood infection has been generally assumed to denote recrudescences of persisting blood parasites and not true relapses, as defined earlier in this thesis. Thus, Schmidt (1953) reports a series of recrudescences occurring at intervals of 17 to 28 days with intervening negative thick films in blood-induced *P. cynomolgi* infections. During the intervening periods when thick films are negative the blood is infective. Further, in sporozoite-induced cases intervening periods occur during which the blood is not infective, and the reappearance of parasites after such a period is termed a relapse.

Corradetti and Verolini concluded that relapses are caused by persisting erythrocytic forms. The work, however, merely shows that recrudescences are so caused, and this is inherent in the definition of a recrudescence. The true relapse may be studied only in sporozoite-induced infections.

Another school of thought in Italy allows that e-e forms are the cause of relapses but that such e-e forms are to be found in R-E cells (Raffaele, 1951, 1952 ; Conti and Monaco, 1950, 1951 ; Gramiccia, 1948). This has been to some degree supported in other Continental countries (Blanc and Languillon, 1949 ; Gallais *et al.* 1949 ; Decourt, 1949b ; Blanc and Cros, 1952 ; Martinez Mujica, 1948). Decourt (1949a) has put forward what he has termed the " French concept " of e-e develop-

ment in all plasmodia and the relation between the described stages and relapse and immunity. He postulates the following stages of development :

1. The inoculation of sporozoites.
2. Nuclear division of the sporozoite within 30 minutes to form protozoites.
3. The protozoites divide by binary fission in mononuclear cells of the connective tissue.
4. Most resultant protozoites undergo a schizogony phase to produce merozoites, which invade erythrocytes. A few protozoites remain in connective tissue cells.
5. Multiplication of the erythrocytic forms.
6. Immunity commences to eradicate blood parasites. Schizonts commence to produce merozoites destined to become gametocytes.
7. Some blood schizonts rest at the pre-segmenting stage. Some schizonts are engulfed by the activated R-E system and there become metazoites by ceasing activity but resisting destruction by the phagocytes.
8. At the time of relapse, three separate processes occur : (*a*) The arrested " dysgonic " stages in the blood resume schizogony. (*b*) The metazoites are released from the R-E cells. (*c*) The few remaining protozoites undergo schizogony and invade the blood.
9. 5 and 6 re-occur.

None of this theory was supported by experimental data.

The theory would appear to take its being from the worst of almost every postulation made to explain relapses and sporozoite development. Although both parthenogenesis and conjugation were overlooked, the theories attributable to Golgi, Bignami, Danilewsky, Missiroli, James and Raffaele may all be detected in this extraordinary synthesis. Not the least surprising fact about the compilation is that selection has been made of the specifically unproven notions of each hypothesis. Protozoites and metazoites have not been shown to exist. No binary fission has been shown to occur in the Haemosporidiidea. Finally, not only is the theory based upon the unproven and undemonstrated, but no account is taken of the established phases of e-e schizogony in mammalian malaria.

It is widely accepted that relapses are occasioned by the breakout of the parasite from a schizogony site in the tissues. It is also widely accepted that this site is in the parenchyma cells of the liver. It is time then to study the clinical and statistical data associated with true malarial relapses in order to obtain some idea of the state of the parasite causing the syndrome.

The following general information is available about sporozoite-induced infections :

(1) *P. falciparum* displays no true relapses (see fig. 1.)
(2) *P. vivax* displays two true relapse patterns (*a*) the so-called tropical zone benign tertian malaria with a haphazard short-term relapse pattern (see

fig. 20), (*b*) the so-called temperate zone benign tertian malaria with a constant long-term relapse pattern (see fig. 20).

(3) *P. ovale* displays true relapses, probably of the short-term variety.

(4) *P. malariae* displays true relapses of varying character but of exceptionally long duration. Reports of relapses occurring up to 42 and 60 years after infection have been made.

(5) *P. cynomolgi* displays true relapses of the 2 (*a*) type according to Schmidt and Genther (1953). I have noted a few relapses occurring between 100 and 110 days after infection, which may or may not be significant, indicating as it does a constant pattern. Schmidt (1953) is quite emphatic about the short-term haphazard relapse pattern of *P. cynomolgi* and, as he has meticulously examined some 1,500 infections, I accept his views.

(6) Persisting e-e forms of *P. cynomolgi* have been found at the time of relapse.

From these data some reasonably firm postulates may be drawn.

1. The tissue phase of *P. falciparum* ceases after the first generation and all merozoites produced by this generation are haemotropic. This is supported by the negative findings in the many thousands of liver autopsies performed *post mortem* on persons who have died from this infection.

2. The tissue phase of *P. malariae* is highly persistant and apparently represents a biological compensation for the hazards of its long cyclic development in the definitive host and its low merozoite production in the intermediate host.

3. The short-term relapse of *P. vivax* and *P. cynomolgi* and probably *P. ovale*, is caused by the persistant e-e phase and the parasites described at the time of relapse in *P. cynomolgi* infections are the result of continuous and active schizogony generations re-invading parenchyma cells of the liver. This is the opinion of such authorities as Shortt, Garnham, Fairley, Boyd, Coatney, Taliaferro and Sergent.

Short-term relapses in untreated *P. vivax* infections have been described from the South-West Pacific by Craige *et al.* (1947) and Coatney, Cooper and Young (1950) [Chesson strain] and from New Guinea by Fairley (1947). It has been assumed by the authorities mentioned that relapse is the result of some temporary recession of immune reactions allowing an establishment of an erythrocytic infection from the constantly menacing e-e schizogony in the liver. This assumes the constant production of haemotropic and some histiotropic merozoites in the liver, and during negative blood phases the haemotropic merozoites are eliminated probably *in situ* by Kupffer-cell action while still free or during their first generation in erythrocytes. Thus, patent infections are disallowed until humoral and cellular activity, particularly in the liver, is for some reason temporarily in abeyance. This theory is best advanced in papers by Shortt and Garnham (1948e) and Fairley (1949). Though these postulations can be accepted in principle, certain modifications will be made later. Garnham (1951a) has suggested that such may not be quite the case in *P. cynomolgi* infections, where immune reactions may not be sufficiently supreme to prevent a continuous or occasionally discontinuous sub-patent

NUMBER OF ATTACKS

WEEKS FROM EXPOSURE TO ONSET OF ATTACK

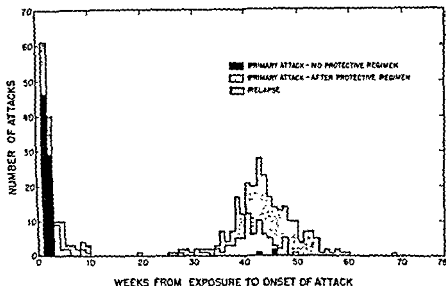


FIG. 20. The two charts display the relapse patterns of a short-term relapse strain of *P. vivax* (Chesson) and a long-term relapse strain of *P. vivax* (St. Elizabeth). (From Coatney, G. R., Cooper, W. C., and Young, M. D., 1950. *J. Nat. Mal. Soc.*, 9, 381, and Coatney, G. R., Cooper, W. C., Ruhe, D. S., Young, M. D., and Burgess, R. W., 1950. *Amer. J. Hyg.*, 51, 200.)

erythrocytic infection constantly renewed from the e-e cycle. Schmidt (1953), however, has reported negative inoculation results at periods of parasitic quiescence in the blood. It may be that this will be no more than a relative question, being a function of the sporozoite density, for, despite Schmidt's finding, many other factors point to a degree of relative commensalism, or better, less damaging parasitism, in the case of *P. cynomolgi*.

It is the long-term relapse *P. vivax* strains which provide the great stumbling block to logical biological inference from observed clinical data. Such strains are to be found in Madagascar (Yorke, 1925; James, 1926; Shute, 1946), the Cameroons (Shute, 1946), the U.S.A.—St. Elizabeth and McCoy strains—(Mayne, 1937; Boyd and Kitchen, 1944; Coatney and Cooper, 1948b; Coatney *et al.*, 1950), the Netherlands (Schüffner, Korteweg and Swellengrebel, 1929; De Buck, 1936; Swellengrebel and De Buck, 1938), Germany (Kikuth, 1943) and in Korea, North Africa and India. They have been generally described in Europe and elsewhere by Hackett (1937), Gill (1938) and Höring (1947). The general pattern of sporozoite infections by these strains is thus: The primary parasitaemia may or may not occur normally and if it occurs the blood eventually becomes negative, owing to the intervention of drugs or the host's immune processes. The blood remains parasite-negative, as has been shown by inoculation for about 40 weeks after infection, until at about this time a clinical relapse occurs. The relapse never occurs shortly after the primary attack as in the short-term relapse pattern of *P. vivax* strains (see fig. 19). The relapses are confined to a period from 20 to 50 weeks after sporozoite inoculation, with a mean at 35 to 42 weeks for the strains studied. Subsequent to the long-term relapse a series of short-term relapses occur for a period of 4 months or so (Coatney *et al.*, 1950 and Shute, 1946). The relapse timing is to some degree dependent on sporozoite loading being an inverse function, but no matter how heavy the sporozoite dosage, the essential long-term mechanism persists (Coatney *et al.*, 1950). This is in contrast to the Chesson short-term relapse strain, where heavy sporozoite dosages bring the relapses on to the very heels of the primary attack (Craige *et al.*, 1947). In addition to this, there is the associated phenomenon of long-term latency in these strains. (It is even the rule in the Dutch strain.) Under the conditions referred to on page 39, a delayed primary attack occurs within the established time range of relapse. It may be safely assumed that the biological mechanism is the same in these twin phenomena of long-term relapse and long-term latency and that the intervention of a normal primary parasitaemia is incidental to the underlying biological cause of the clinical occurrence.

This pattern is plainly a phenomenon associated with the parasite itself and the pattern must have as its genesis a definitive biological parasite mechanism. The day-to-day timing of the relapse may owe something to the state of the host but the general period of relapse probability is obviously dictated by innate biological qualities of the parasite.

There are four possible biological answers to this problem, based on the existence of persisting e-e forms:

1. Some pre-e schizonts cease activity before merozoite release and become latent. These forms are reactivated by some intrinsic mechanism at the set time and this reactivation is independent of the immune status of the host (Boyd, 1953, and after Shute, 1946).
2. While the majority of sporozoites invade liver cells and actively grow there, some sporozoites do not develop but assume a latent and inactive state. This may occur in parenchyma cells, in R-E cells, in connective tissue cells of the skin, in the lymphatics or extracellularly (after James, 1931a and Shute, 1946). Alternatively, sporozoites travelling by the lymph system may reach the liver intermittently and after long delays (Griffiths and Gordon, 1952)
3. A general slowing down of e-e schizogony rates combined with the production of low numbers of haemotropic merozoites and high numbers of histiotropic merozoites causes a low density of erythrocyte-invading merozoites, which is easily dealt with by the immune responses. At the set time, the haemotropic/histiotropic ratio becomes high once again and schizogony is speeded up to provide a threshold number of haemotropic merozoites sufficient to overcome the low premunition of the host (Fairley, 1949).
4. A complete change over to histiotropic merozoite production occurs on the part of the e-e cycle. At the set time normal haemotropic/histiotropic ratios are resumed in the relative absence of immune reaction (Fairley, 1949).

Such changes within the e-e cycle as are postulated in 3 and 4 are adaptations on the part of the parasite to ensure continuance of infection in areas where mosquitoes hibernate.

Some remarks have already been made about the first of these explanations to the effect that arrested schizonts have not been seen. The theory has been put forward by Boyd (1953) to explain a supposed differential action of pyrimethamine which in fact does not exist (Coatney *et al.*, 1953). The theory is still held by Shute, who likens this development to similar arrest in sporogony which, as he has informed me, he has noted up to 3 months after infection of anopheles. Much as I have relied and will continue to rely upon analogies with sporogony, I now part company with such comparisons. Shute has obtained such arrests of sporogony only by virtue of maintaining the anopheles at temperatures lower than 21°C. Subsequent sporozoite development is uncertain. It is difficult to conceive of similar events affecting the e-e cycle in man *as a rule*, for relapses are the rule, not the exception. A further point by way of an *obiter dictum* is that this theory may be used to explain relapses as a whole but then does not explain the

differences between the patterns, or may be used to explain the long-term relapse but then does not explain the series of subsequent short-term relapses. Unfortunately I have been unable to find an estimation of the average life of a parenchyma cell as this surely must be an important point. Death of the host cell must spell death to the enclosed parasite. It seems that it would be unlikely that a sufficient number of arrested schizonts would inhabit parenchyma cells capable of maintaining the parasite for the necessary 9 months.

As to the second suggestion, although sporozoites have been seen in lymph glands 24 hours after inoculation (Boyd and Kitchen, 1939) and they have been known to survive in defibrinated blood for up to 5 days (Mayne, 1933), they normally quickly degenerate in most tissue or body fluids outside the body. On the other hand, they can overwinter in a mosquito's glands. However, from general considerations it seems doubtful if a sporozoite would survive long extracellularly in the body of a vertebrate at 36·9°C. If intracellularly, by what mechanism is it released at some definite date? Most cells would precipitate the sporozoite into the general system at irregular intervals as these cells break down. Though little definite can be said about this theory, it must be viewed unfavourably.

It is the choice between the third and fourth explanations which presents such insoluble problems. Let it be said at the outset, for it will soon become painfully apparent, that there exists no satisfactory explanation for long-term relapses and little hope is discernible for the future as overriding objections appear in every circumstance postulated. It is at this point where chemotherapeutic considerations come forward, but, alas, to cloud the issue, not to clarify it. The first of these two explanations is favoured by the postulation already made of resistant retarded forms insensitive to proguanil as being the organisms causing short-term latency in proguanil-treated infections. It must be remembered that proguanil therapy has no effect upon the relapse rate of either pattern of *P. vivax* once therapy has ceased. That is to say, proguanil fails to cause radical damage to the persisting e-e forms. On the other hand, Hawking and Thurston showed it to have some effect, and in fact they were unable to demonstrate pre-e forms after full proguanil treatment. Thus, if the postulate concerning retarded forms is accepted then it must be further accepted that these forms are able to continue the e-e schizogony and at the same retarded proguanil-resistant rate in order to survive. This is necessary as it is not believed that pre-e and e-e forms differ in any manner which is chemotherapeutically detectable. This, in other words, means a slowed-down schizogony rate and a high histiotropic/haemotropic ratio to maintain e-e infection in the face of both proguanil attack and initially low retarded schizont proportions. In the absence of proguanil it would be assumed that immune responses force a similar type of development as has been postulated already in *P. gallinaceum* infections.

Were it not for Hawking and Thurston's observation it would be simpler to

maintain that the so-called prophylactic effect of proguanil; and indeed pyrimethamine, was nothing more than a residual action against sub-patent primary erythrocytic infections and adopt the second of these two explanations, as is favoured by Fairley (1949). Certainly it is the more biologically acceptable. In this case, histiotropism is forced upon the parasite by an inimical climate and an innate characteristic calls it back to haemotropism at a given time. But these forms should be open to some attack by proguanil, such attack growing in force as the number of actively growing schizonts diminishes. Drug resistance is not developed in the e-e infections of a single host so this does not explain the inefficacy of proguanil.

At this stage it seems expedient to ignore the action of proguanil, assuming it to have some effect upon the e-e cycle not yet explicable, and to concentrate upon a general postulation concerning the persisting e-e phase in the genus *Plasmodium*. This contention is that a normal basic haemotropic/histiotropic merozoite formation ratio exists for any one plasmodium. This may be 100/0 as in *P. falciparum* or about 99/1 as in *P. vivax* or about 50/50 as in *P. gallinaceum* or *P. elongatum*. This ratio may be upset by a variety of circumstances. Thus, it may be altered by drug action as in quinine treatment of *P. gallinaceum*, or by immune reactions as in short-term relapse *P. vivax* strains, or by selection of a different ratio pattern as in long-term relapse *P. vivax* strains. In this last case, the variation has been selected and has become a fixed characteristic of the strain. Dealing with particular examples, we have an increase of histiotropic/haemotropic ratio as the infection of *P. elongatum* progresses, due to the increasing immune response to the host. We have an increased histiotropic/haemotropic ratio in the parasite-negative blood phases of the short-term relapse *P. vivax* infections caused by the host's immune reaction. On any weakening of the host's immune reaction normal histiotropic/haemotropic activity is resumed. Finally, in the case of the long-term relapse *P. vivax* strains, we have a strain selected as a result of environmental stresses, which now displays certain fixed characteristics. These are a normal histiotropic/haemotropic ratio followed by an exclusive histiotropic merozoite production for a fixed time, after which normal histiotropic/haemotropic characters resume their sway. This is followed by the usual reaction to immunity factors as displayed by short-term relapse strains.*

It will be noticed that some modification of the theories of Shortt and Garnham and of Fairley concerning short-term *P. vivax* relapses has been made. This has been made not only to fit a general postulation but also to provide some way out of the dilemma raised by immunity considerations. If a watchful immunity complex is destroying haemotropic merozoites and sub-patent erythrocytic infections as they arise it seems that the small number of histiotropic merozoites are in great danger and that the sharp drop in numbers from first to second generation e-e forms should continue until the cycle is eliminated. If, however, an in-

* See footnote on page 39.

creased number of histiocytic merozoites is produced, this would go some way towards ensuring a persistence of the e-e cycle. There remains, *of course*, the argument that immune responses are specific to haemotropic merozoites or, more likely, only to the merozoite once it has invaded a red cell.

Before ending the discussion on relapses, it must be stressed that, though certain theories are advanced here it is not to say that they are held with either comfort or great conviction.

Immunity

Acquired Immunity

There is at present only one observation upon acquired immunity to the e-e stages of mammalian malaria parasites. It was found by Shortt and his colleagues that no acquired immunity to pre-e forms of *P. vivax* occurs in the one case studied by them (Shortt, Garnham, Covell and Shute, 1948 ; Shortt and Garnham, 1948c ; Shortt, 1951a). A human volunteer, who had undergone sporozoite-induced *P. vivax* malaria therapy some 22½ months previously, was subjected to massive sporozoite inoculation and pre-e forms were demonstrated in his liver. On the other hand, no overt parasitaemia was detectable and no clinical attack ensued. Thus it was clearly shown that, while a powerful residual immunity to the blood forms was still active, no such immunity existed to pre-e forms.

This observation bore out general inferences made over a number of years by numerous workers including Boyd (M. F.), Coatney, Russell, Covell, Mulligan, Swellengrebel, Paraense and others. The very fact of the relapse phenomenon denotes a lack of an effective immune response to e-e forms. Shortt and Garnham (1948c) noted that superinfection by sporozoites is possible in *P. cynomolgi* infections, denoting a lack of specific immune reaction to pre-e forms. Boyd (1947) found that the specific immune response to *P. vivax* infections *was directed solely* against the erythrocytic forms and not against the sporozoites and their immediate descendants.

Inevitably, from the discussion on relapses already recorded, I cannot take *up the position that there is no reaction on* the part of the e-e cycle to general immune reactions of the host. It has already been postulated that acquired *immunity may have an effect* upon the mode of e-e development. I persist in this belief in the light of the results obtained by Shute (1946) from cross-immunity *experiments using the long-term relapse P. vivax* strain from Madagascar and the long-term relapse *P. vivax* strain from the French Cameroons. Shute described delayed primary attacks up to 300 days delayed, in sporozoite infections of the French-Cameroons strain of *P. vivax* in patients previously immunized against the Madagascar strain of *P. vivax*. This would appear to be an example of immune reactions modifying the e-e development of a parasite, which normally *would* probably have displayed the usual primary attack followed by a long-term relapse. This could, of course, have been purely the result of immunity *attack on the*

erythrocytic phase but there is no reason to believe that the normal e-e pattern has not also been disturbed. On the other hand, it is stressed that only the mode of development is affected, and more severe effects are absent. It is also stressed that the action may well be only against the extracellular progeny of the e-e phase and not the intracellular schizogony stages proper. This last is supported on general microscopic evidence which shows no micro-reaction to the intracellular stages with the exception of *P. knowlesi*, and only the progeny following the bursting of the schizont excite the usual infiltration reaction.

Natural Immunity

The only specific recorded evidence on natural immunity to e-e forms concerns attempts to infect the liver of *M. mulatta* with sporozoites of *P. vivax*. This has been attempted by direct inoculation by Huff and Coulston (1948) and Hawking and Thurston (1952). Only the latter attempt was thorough and was, unfortunately, completely negative. Similar failure can also be reported in attempts to infect day-old mice with large numbers of sporozoites of *P. cynomolgi*. Numerous attempts have been made to infect various unnatural hosts with the sporozoites of mammalian plasmodia. In the numerous failures there may be many cases of natural immunity to pre-e forms but in the light of the evidence of similar avian malaria studies this is not certain and no conclusions can be drawn.

Pathogenicity

As far as can be judged, the e-e forms, as such, of mammalian plasmodia, with the exception of *P. knowlesi*, exert no pathogenic effect upon their intermediate hosts other than the destruction of a negligible number of parenchyma cells and a consequent minor local infiltration. *P. berghei* may also prove an exception when its full development is known. In *P. cynomolgi* infections, monkeys having as many as 20,000 schizonts per cm.^{3} of liver, show no untoward symptoms and it is only when the blood is invaded that any signs of illness appear.

The described e-e stages of *P. knowlesi*, however, display a ring of reaction about them and scar tissue has been described in the liver resulting from burst or degenerated schizonts. Thus, some real measure of pathogenicity occurs. In general, it can be said that, like the sporogonic stages, the e-e cycle of mammalian plasmodia causes no discomfort to the hosts and it may yet be shown that the large schizonts of *P. knowlesi* are degenerate forms and are, by a small stretch of the imagination, analagous to the black spores of Ross.

(*d*) GENERAL CONSIDERATIONS; RELATED GENERA

General Considerations

There remain a number of lacunae in the general knowledge of the mammalian e-e cycles and a number of " loose " facts which seek a place in the schemata of

e-e schizogony patterns. Firstly, more knowledge is necessary *along the following lines* :

1. Study of the pre-e and e-e stages of *P. berghei* or *P. vinckei* and the early pre-e stages of *P. knowlesi* is necessary to complete the knowledge of possible patterns of mammalian e-e schizogony. The possibility that the rodent malarias provide the link between mammalian and avian e-e schizogony must be explored.
2. It would be of great assistance if some morphologically distinct e-e form of, say, *P. cynomolgi* or *P. vivax*, which could be related to histiotropism or the *long-term relapse pattern, could be distinguished*, but one can *hold* out no great hope for this. It is believed that the tropism is more likely to be of a chemical nature than microscopically demonstrable.
3. The immediate fate and reactions of the sporozoite need to be studied. Such questions as the following require definite answers : What proportion of the sporozoites in a mosquito's glands are inoculated? What proportion reach the liver and by what routes? *Are there any stragglers or any which travel successfully and enter liver cells by routes other than* the blood? Does the sporozoite require any activation to cause it to invade the inactive parenchyma cell?
4. The most important investigations necessary involve the fundamental biochemical and physiological aspects of the e-e stages. Such studies may provide data on the genesis of relapses and may open up new routes of attack upon the parasite, which are badly needed. *Data of this kind must* come from studies on the parasite itself as well as from indirect sources.

There is an amount of " loose " knowledge which requires firm dovetailing with the scheme of knowledge as it stands at present. Such facts are the effects of proguanil therapy, the retarded forms of *P. cynomolgi*, and the minor morphological and biochemical differences between *P. cynomolgi*, *P. inui* and *P. falciparum*. One outstanding fact is not yet correlated. Why is the liver favoured as the site of e-e schizogony? Has the bile any significance? Is there any helpful distribution within the organ as a whole? A thousand other minor questions arise from scraps of unintegrated knowledge.

There is one point which I would *like to insert here. This is the question of controls as it affects all the work done on the* mammalian pre-e forms. Huff (1950, 1953a) has listed, among other objections to the findings of Shortt and Garnham, the lack of controls in the various experiments. In a private communication he has contended that such processes as have been described *in the variously infected mammals may be artefacts, though of what nature he has not expressed an opinion.* Coulston and Robinson (1950) have gone so far as to infer that the pre-e forms of *P. cynomolgi* described are, in fact, agglutinated platelet aggre-

gations. This last suggestion may be dismissed; such aggregations do occasionally occur in liver sections but they are quite distinct from pre-e forms. They are of irregular shape, all sizes, and stain a uniform pink.

As to the criticism concerning controls, it must be recorded that control experiments have been performed both by Professor Shortt (Shortt, Bray and Cooper, 1954) and by Professor Garnham, involving *P. cynomolgi* and *P. inui* respectively. Control monkeys were subjected to exactly similar techniques, but the mosquitoes were fed on clean monkeys and had no gland infections. All liver sections from control monkeys were negative. Perhaps more interesting were the scientific ethics involved in the *P. cynomolgi* experiment and the unrecorded details of the experiment will be briefly described here.

At the same time as another experiment was being performed, largely by me, Professor Shortt and Mr. Cooper repeated the techniques, using clean monkeys. This was done without my knowledge. Liver was taken at autopsy 7 days later from the control monkey and was sectioned and stained. At this time the experiment in which I was involved had proved successful and pre-e forms were demonstrated. Some time later Professor Shortt brought me 300 sections of liver and asked me to examine them for 7-day-old pre-e forms of *P. cynomolgi*. Professor Shortt was never renowned for lengthy explanations of his every action and his somewhat laconic question caused no surprise. I assumed the sections to be some unsearched material from some earlier experiment. Some four weeks later, and even with a little annoyance at what I had thought was a wasted 10 hours or so at the microscope, I returned the slides to Professor Shortt with the statement that they were completely free from pre-e forms. It was then that I was told that I had searched about 300 sq. cm. of liver from the control uninfected monkey.

Many references have been made in this chapter to the analogies to be drawn with the sporogony cycle. A comparison of growth rates on the basis of diameters is made in fig. 21. This comparison is most striking and in entire accord with the findings of Huff (1954) in avian malaria, in fact, the similarities between e-e schizogony and sporogony (other than gametogony) are even more striking in simian malaria parasites than in the avian malaria parasites. Huff went on to say that the two cycles are for material purposes similar and schizogonic. There is, however, another side to this question. Elsewhere I have mentioned the general similarity of morphology and mode of growth between sporogony, microgametogony and schizogony in the Coccidiomorpha (Bray, 1954). In certain genera, such as certain members of the Eimeriidae, the similarity between schizogony and microgametogony is most striking and only in the final product is any great difference discernible. Even among the avian plasmodia, where Huff points to the similarities between e-e schizogony and sporogony, a similar comparison could be made between erythrocytic schizogony and microgametogony of, say, *P. elongatum* with almost as much justice. Such similarities should be taken as

evidence of a similar mode of growth throughout the whole sub-class but should not confuse the biological meaning of each growth phase.

Such overall considerations *do not by any means invalidate* Huff's contention for there can be no doubt that his conclusion is true and is, *in fact, axiomatic.* If we regard the genus *Plasmodium* as largely of a diploid character and accept

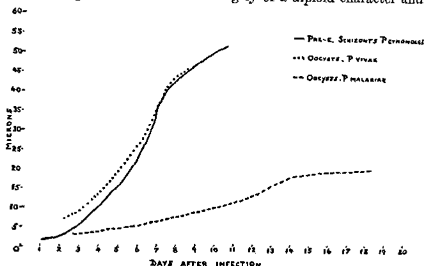


FIG. 21. Curves showing the growth rate (size/time) of the pre-e schizogony stages of *P. cynomolgi* and the sporogony stages of *P. vivax* and *P. malariae.* Sizes are shown as diameters in microns. It can be assumed that the growth rates of oocysts of *P. vivax* and *P. cynomolgi* are almost the same. It will be seen then that growth rates for the pre-e schizogony phase and the sporogony phase are almost the same. *If the original infecting organism is taken into account, i.e., the 25 × 10μ* oökinete and the 12 × 1μ sporozoite, it can be seen that the growth rates are even closer in relation than is apparent from the graph. This and various other similarities, notably the identical sensitivity to drugs, and the number of daughter parasites produced, makes comparison between these two cycles inevitable. The growth rate curve of *P. malariae* is displayed to show the tail-off of apparent growth rate after release of sporozoites has commenced. *A similar tail-off is apparent in the P. cynomolgi* pre-e stage growth rate curve. In both cases this is due to late maturing retarded forms giving an apparent decrease in average diameter after normal forms have disappeared.

that reduction division to haploid character occurs during gametogony, *i.e.* during the production *of microgametes and the throwing off of* "polar bodies" by the macrogametocyte, then the true extent of the sexual phase is delineated by the phase of haploid characteristics. This commences with gamete formation and ceases with fertilization.

If on the other hand we treat *Plasmodium* as largely haploid (by analogy *with Aggregata*) then the sexual phase is the diploid *phase which ends with the first division of the zygotic nucleus.* Thus the logical conclusion is that all nuclear divisions during sporogony, with the exception of the first, are mitotic and essen-

tially asexual. Thus schizogony and what we know as sporogony are asexual and fundamentally identical, whether it is believed that gametic meiosis or zygotic meiosis is the case in *Plasmodium*.

The widespread notion that the whole of the phase of *Plasmodium* occurring in the arthropod is sexual is, in the strict sense, nothing more than the old mistake of arguing from the particular to the general for the sake of convenience. Merely because some of the stages in the mosquito are sexual it has been widely assumed that all the stages in this host are sexual, which is palpably untrue.* Volume comparisons between the two cycles yielded no further information, but a comparison of the approximate volumes of mature e-e forms is listed below for information :

P. gallinaceum in R-E cells	1,400 cu.μ	or	$1{\cdot}4 \times 10^{-9}$ cm.^{3}	by formula calculation
P. elongatum	175 cu.μ	or	$1{\cdot}75 \times 10^{-10}$ cm.^{3}	by formula calculation
P. inui	7,000 cu.μ	or	7×10^{-9} cm.^{3}	by formula calculation
P. cynomolgi	33,000 cu.μ	or	$3{\cdot}3 \times 10^{-9}$ cm.^{3}	by formula calculation
P. vivax	50,000 cu.μ	or	5×10^{-8} cm.^{3}	by formula calculation
P. falciparum	88,000 cu.μ	or	$8{\cdot}8 \times 10^{-8}$ cm.^{3}	by direct measurement

and for comparison

H. kochi
merocyst up to 32,000,000,000 cu.μ or $3{\cdot}2 \times 10^{-2}$ cm.^{3} by formula calculation

Related Genera

At one time or another the following species have been described as belonging to the genus *Plasmodium* in the family Plasmodiidae.

SPECIES	HOST
semnopitheci	Langur
kochi	African apes
limnotragi	Antelope
foleyi	Lemur
vassali	Squirrel
brodeni	Elephant shrew
pteropi	Fruit bat
epomophori	Fruit bat
murinus	Insectivorous bat
melanipherus	Insectivorous bat

* The action of mitotic poisons such as sulphonamides, proguanil and pyrimethamine is interesting in this context. These drugs are without apparent effect upon the divisions of nucleus involved in gamete production but immediately arrest the first zygotic division (Terzian, Stahler and Weathersby, *1949; Shute and Maryon, 1948, 1954*). Does this mean that these drugs are without effect upon meiosis and that the enzyme complex which they inhibit is not required in the reduction division of nuclei, and further is it therefore proven that *Plasmodium* undergoes gametic meiosis unlike *Aggregata*? It would be useful in this connexion to know the precise action of sulphonamides on each stage of *Eimeria* where it is thought that zygotic meiosis occurs.

Other parasites such as *bouilliezi*, *cephalophi*, *cercopitheci*, *joyeuxi*, *mackiei*, *ratufae*, *rigolleti*, *roubaudi*, *taiwanensis* and *tyrio* are at present treated as synonyms of one or the other of the species listed above. These parasites will be briefly discussed in conjunction with the similar parasite *Nycteria medusiformis* Garnham and Heisch, 1953. None of these parasites displays schizogony in the blood, nor have any of them been transmitted by *Anopheles*. They are patently not plasmodia and, in fact, they display the characteristics of the Haemoproteidae. It is to this family that they belong.

Garnham (1948a, 1948b, 1950a, 1950b, 1951a, 1951e, 1953a, 1953b) has been responsible for the reclassification of these parasites and their arrangement within existing and new genera of the family Haemoproteidae. This has largely been



Fig. 22. Merocyst of *Hepatocystis kochi* in liver section. Stained with Giemsa-colophonium.

done on the basis of the parasite schizogony which is considered by Garnham to be a more fundamental characteristic than the gametogony in the blood. The e-e schizogony is known in the following species :

Species	Type of Schizogony
kochi (see fig. 22)	Merocysts in liver parenchyma
vassali	Merocysts in liver parenchyma
epomophori	Merocysts in liver parenchyma
murinus (see fig. 23)	Very small schizonts in endothelial cells
N. medusiformis (see fig. 23)	*Cynomolgi*-type schizonts in liver parenchyma

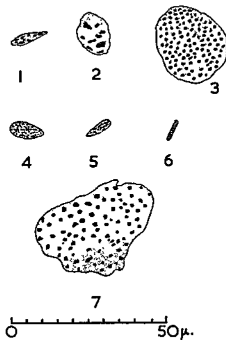


Fig. 23. Exo-erythrocytic schizogony of mammalian Haemoproteidae.

1, 2 and 3. Immature schizonts of *Polychromophilus murinus* in a liver smear. Stained with Giemsa. 4. Mature schizont of *P. murinus* in a liver section. Stained with haematoxylin and eosin. 5 and 6. Immature schizonts of *P. murinus* in a liver section. Stained with haematoxylin and eosin. 7. Immature schizont of *Nycteria medusiformis* in a liver section. Note the tendency to nuclear segmentation. Stained with Giemsa-colophonium.

The *kochi* type of schizogony has been briefly referred to and on this basis Garnham (1948a) removed the species to a new genus *Hepatocystis*. These observations on *H. kochi* have been confirmed by Haddow (1952) and expanded by

Garnham and Pick (1952). The similar schizogony of *vassali* was described by Field and Edeson (1949a, 1949b) and Edeson and Field (1951) who removed this species to the genus *Hepatocystis*. Ray (1949) described large parasitic processes in the liver of a squirrel infected with *vassali*. The schizonts resemble those described in *P. knowlesi* infections and, although only gametocytes appeared in the blood, Ray declined to remove the parasite from the genus *Plasmodium*. Garnham (1950a, 1950b, 1951e) and Rodhain (1953) have described merocystic schizogony in *epomophori* infections of the liver of bats. Thus, there are three species in the genus *Hepatocystis*. Garnham (1948b, 1951a, 1953b) has suggested that they will probably be joined by *semnopitheci*, *limnotragi* and *pteropi*. To these *foleyi* should be added.

The e-e schizogony of *murinus* has been noted. Garnham (1951a, 1953b) has suggested that this species and also *melanipherus* should be replaced in the genus *Polychromophilus* given them originally by Dionisi (1899). Garnham has suggested that *brodeni* may belong in this genus. Finally, a new parasite *N. medusiformis* has been shown by Garnham and Heisch (1952, 1953) to display only gametocytes in the blood and a *cynomolgi* type of schizogony in the liver. There are differences from *P. cynomolgi* schizogony in the appearance of the nuclei.

These three genera have been assigned to the Haemoproteidae by Garnham (1953a) and the only point of real doubt is the position of *brodeni* and, possibly *pteropi*. As in the case of the mammalian malaria forms and *Leucocytozoön*, it has proved impossible to transmit *H. kochi* by inoculation of liver or individual merocysts.

(ε) DIFFERENCES FROM AVIAN FORMS

There still exists some confusion owing to the prior demonstration of the avian e-e forms and it has been thought useful to compile a list of essential differences between the avian e-e forms and the mammalian e-e forms and also between the related phenomena existing in the two groups.

Avian Malaria	Mammalian Malaria
Related Phenomena	
1. Various tissues in sporozoite-induced infections are infective.	Similar tissues in sporozoite-induced infections are not infective.
2. Schizontocides are not curative in blood-induced infections.	Schizontocides are curative in blood-induced infections.
3. The host may die from a sporozoite-induced infection before appreciable multiplication of the blood forms.	Only the blood forms produce a reaction from the host.
Directly observed phenomena	
1. E-e forms are easily demonstrable in sporozoite- and blood-induced infections.	E-e forms are difficult to demonstrate in sporozoite infections and are undemonstrable in blood infections.

2. E-e forms increase progressively in the early stages.	E-e forms decline sharply in number after the first generation.
3. E-e forms are found in endothelial or haemopoietic cells all over the host's body.	E-e forms are found exclusively in epithelial cells in the liver.
4. Merozoites number up to 1,000.	Merozoites number never less than 1,000.
5. One schizogony generation occupies not more than 48 hours.	One schizogony generation occupies at least 5 days.
6. E-e forms excite a definite histologically demonstrable response and are pathogenic.	E-e forms excite no cellular reaction.
7. Two pre-e generations occur.	One pre-e generation occurs.

One similarity should be stressed in the light of the objections raised by Huff (1950 and 1953) who has stated that the stain reaction of the pre-e forms of *P. cynomolgi* differs from that of the pre-e and e-e forms of the avian plasmodia. This impression should be sharply corrected. Huff has used a haematoxylin stain which has not been accepted as a general stain for *Plasmodium* and, on his own admission, stains parasite nuclei poorly. On the other hand, the Romanowsky stains used by Shortt, Garnham and others are universally accepted as specific for *Plasmodium* and other haematozoa. My personal experience has been that the stain reaction to the Giemsa-colophonium technique is identical whether avian or mammalian blood or tissue forms are examined in section.

CHAPTER 6

TAXONOMY AND SPECIFIC CLASSIFICATION

THAT the taxonomy of the Haemosporidiidea would be affected by the discovery of the e-e cycle of *Plasmodium* was axiomatic. The contentions, as to the necessary changes, of Missiroli, James, Corradetti, Manwell, Gramiccia, Verney and Giovannola have been noted. The work of Garnham has removed various species from the Plasmodiidae to the Haemoproteidae. It has already been stated that it is thought more politic to increase genera and decrease species than *vice versa.* This view has its important practical considerations. To have a well defined mode of development within a genus is of great value to a student. To have a genus include a number of differing modes of development can be most confusing. For these reasons, any plan to fuse the Plasmodiidae and the Haemoproteidae is rejected. Such a plan would include in one family megaloschizonts in the spleen and microschizonts in haemopoietic tissue, gametogony only in the blood and both gametogony and schizogony in the blood, various arthropod vectors and exclusively mosquito vectors. It would seem wasteful to have so many well differentiated characteristics available, sufficient in higher phyla surely to differentiate between orders, and not at least to maintain those families at present recognized, if not to increase them.

As to the Plasmodiidae, there is some basis for further differentiation into more than one genus. It is useful to list a number of groupings within the genus *Plasmodium* and to devise from such a list a possible revision of the Plasmodiidae. Let it be said that much of the necessary work has already been accomplished by Garnham (1948a, 1951a, 1953a) and his conclusions will be listed later.

1. *Sporogony.* Two groupings may be distinguished on host specificities :
   (*a*) Sporogony occurs exclusively in anophelines ; slightly larger sporozoite : human, simian, rodent and probably chiropteran malaria.
   (*b*) Sporogony occurs in any of the anopheline or culicine mosquitoes ; slightly smaller sporozoite : avian and probably saurian and amphibian malaria.
2. *Erythrocytic schizogony.* Once more, two groupings may be distinguished :
   (*a*) Schizogony takes place in non-nucleated red blood cells ; merozoites are unable to invade and grow in tissue : human, simian and probably rodent and chiropteran malaria.
   (*b*) Schizogony takes place in nucleated red blood cells ; merozoites are able to invade and grow in tissue cells : avian, saurian and probably amphibian malaria.

3. *Exo-erythrocytic schizogony.* Two main groupings may be distinguished but these may be further sub-divided.
   (*a*) Schizogony in epithelial cells; one pre-e generation; mature schizonts nearly macroscopic and containing never less than 1,000 merozoites: human and simian malaria; probably chiropteran malaria; rodent malaria is doubtful.
   (*b*) Schizogony in endothelial or haemopoietic cells; two pre-e generations; schizonts microscopic and containing less than 1,000 merozoites: avian and saurian malaria; probably amphibian malaria.

Thus it is apparent that two main groupings more or less consistently resolve themselves. One group parasitizes the mammalia, the other the aves and their progenitors the sauria.

However, the e-e schizogony allows further differentiation. Garnham (1953a) believes that erythrocytic schizogony is a comparatively recent development from the usual type of tissue asexual schizogony displayed by the various members of the sub-class Coccidiomorpha. Garnham therefore has stated that this basic tissue phase is the more fundamental characteristic and that classification should refer to it. As has been shown, 5 patterns of e-e schizogony exist in the plasmodia. These five patterns, as listed by Garnham (1951a, 1953a), and slightly modified here are:

1. *Falciparum* —Schizogony in liver parenchyma; one generation only: includes *P. falciparum*, and *P. reichenowi*.
2. *Cynomolgi* —Schizogony in liver parenchyma; many generations: includes *P. vivax*, *P. malariae*, *P. ovale*, *P. schwetzi*, *P. pitheci*, *P. inui*, *P. gonderi*, *P. simium*, *P. hylobati*, *P. brazilianum*, and probably *P. girardi* and *P. roussetti*.
3. *Gallinaceum* —Schizogony in endothelial cells: includes *P. gallinaceum*, *P. relictum*, *P. cathemerium*, *P. lophurae*, *P. fallax*, *P. circumflexum*, *P. durae*, *P. juxtanucleare*, *P. hexamerium* and probably *P. pitmani*, *P. polare* and *P. agamae*.
4. *Mexicanum* —Schizogony in both endothelial and haemopoietic cells: includes *P. mexicanum*, probably *P. floridense* and possibly the other New World saurian species.
5. *Elongatum* —Schizogony largely in haemopoietic cells: includes *P. elongatum* and *P. vaughani*, probably *P. huffi* and *P. rouxi*, and possibly *P. nucleophilum* and *P. lacertiliae*.

Such a scheme leaves a number of Old World saurian species and the amphibian species unclassified as information about them is so scanty.

Two important omissions are made. *P. knowlesi*, which present descriptions leave unclassified, may well pose an interesting problem. *P. berghei* may prove a link between avian and mammalian types.

Garnham (1953a) has made these 5 patterns the basis for the suggested creation of 5 genera in the family Plasmodiidae. These 5 genera are respectively:

1. *Laverania* Feletti and Grassi, 1890
2. *Plasmodium* Marchiafava and Celli, 1885.
3. *Proteosoma* Labbé, 1894.
4. Yet to be named
5. *Istiocytozoön** Missiroli, 1937

I am unable to agree entirely with this suggested formulation. As has been noted on page 57, the theory that major differences exist between the types of e-e schizogony displayed by *P. gallinaceum*, *P. elongatum* and *P. mexicanum* is not entirely acceptable. On the other hand, it is believed that wide differences occur between the groups 1, 2 and 3, 4, 5.

A more fundamental consideration is that of phylogeny. It is not possible to be dogmatic about this problem but what can be done is to indicate the most logical and likely postulation on the origins of the genera in question. This postulation is that the ancestors of the family Plasmodiidae were haemogregarine-like parasites.

Among the haemogregarines we can detect the first appearance of forms in the blood stream which facilitate transmission by blood-sucking arthropods. The forms may be gametocytes or sporozoites. It is with the group of haemogregarines belonging to the order Adeleida and producing gametocytes in the circulating blood that we are concerned. The group contains three genera, *Haemogregarina*, *Karyolysus* and *Hepatozoön*. The first appears in amphibia and is therefore probably the oldest; the second is found in reptiles while the last and most recent is to be found in reptiles, birds and mammals. *Hepatozoön* not only inhabits mammals but shows a preference for both parenchyma and the liver unlike the other two genera.

It seems probable that from this group two lines have developed. From the genus *Karyolysus* having a schizogonic development in endothelial cells in the lung, etc. come those genera of the family Haemoproteidae which undergo schizogony in endothelial cells in the lung, etc. This is typified by the genus *Haemoproteus* of reptiles and birds. Also represented here is a further step in parasitism from reptiles to birds. From the genus *Hepatozoön*, having a schizogonic development in parenchyma cells of liver and lung but especially liver in mammals, come those genera of the Haemoproteidae which undergo schizogony in liver parenchyma. This is typified by the genera *Nycteria* and *Hepatocystis*. Also represented is the step from reptilian, avian and mammalian hosts, to purely mammalian hosts commencing with the primitive Chiroptera.

So far this postulation has: (1) omitted all reference to *Leucocytozoön* which

* Should this general formulation be accepted the name *Istiocytozoön* must on no account be used. It is the thoroughly illegitimate offspring of Modern Italian and Ancient Greek and was used in the first place by Missiroli to include several species with divergent host cells.

appears to have a foot in both worlds (schizogony in liver parenchyma and spleen endothelial cells?) and which probably does not concern the present argument; (2) omitted reference to *Polychromophilus* which can be regarded as the first appearance of *Haemoproteus*-like development in lower mammals; (3) detailed a process of phylogenetic development which cuts across the taxonomy of certain authors who have made use of syzygy as a basis of classification.

On this last point most authors have agreed in the designation of two or three sub-classes or orders in the Sporozoa which presently concern this chapter. These two or three groups are the Coccidiida (Coccidia) Adeleida and the Haemosporidiida (Haemosporidia); the Adeleida are often included in the Coccidiida as a sub-group. This formulation is perfectly in accord with the above thesis. However, some authors, notably Wenyon, have provided a classification based on syzygy which groups the Haemosporidia as a sub-order of the Coccidiida and leaves the Adeleida as a separate order. If this view is taken as correct, it seriously challenges the above thesis that the Haemosporidia have evolved from the haemogregarines of the Adeleida. It is because I am convinced that the above view of phylogeny is correct that the classification set out on page 2 has been adopted in preference to that of Wenyon.

Tracing these lines of development into the Plasmodiidae it can be logically argued that the malaria parasites of birds, reptiles and amphibia develop from the line typified by *Haemoproteus* while the malaria parasites of mammals develop from the line typified by *Nycteria* and *Hepatocystis*. This view points to the designation of (*P.*) *mexicanum* as the most primitive fully known parasite in the avian and saurian line. From this source two lines are in the process of formation; the one typified by (*P.*) *gallinaceum*, the other by (*P.*) *elongatum*. It is my belief that these developments are still in a clinal state and not yet sufficiently advanced to be worthy of generic status.

In the mammalian group the most primitive parasite appears to be (*P.*) *knowlesi* with its *Hepatocystis*-like development in the liver. Certainly the most advanced parasite is (*P.*) *falciparum*, not only by reason of its activity in its host but, more important, by reason of its further step in the shift from tissue to blood schizogony.

This postulation demands one of three taxonomic corrections to the existing systems of classification:

1. The fusion of the Plasmodiidae and the Haemoproteidae into one family—the Plasmodiidae.
2. The fusion of the genera *Haemoproteus*, *Polychromophilus*, *Nycteria* and *Hepatocystis* into one genus—*Haemoproteus*, and the retention of the single genus *Plasmodium* in the Plasmodiidae.
3. The formation of two genera (or three) in the Plasmodiidae; one representing the line developing from *Haemoproteus* and one the line from *Nycteria* and *Hepatocystis*.

The first suggestion is contrary to those tenets of biological classification which are based on the type of phylogenetic consideration detailed above. The second suggestion necessitates a belief that *Haemoproteus* and *Hepatocystis* schizogony are essentially similar, which they are patently not, though a case could be made for the fusion of *Haemoproteus* and *Polychromophilus*. The third suggestion appears sound and has morphological differences to support it.

Taking all the facts and arguments recorded in this chapter into consideration, a sub-division of the family Plasmodiidae into two genera is recommended.

1. The genus *Plasmodium** Marchiafava and Celli, 1885.
2. The genus *Haemamoeba* Feletti and Grassi, 1889.

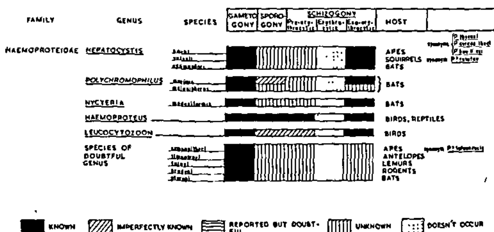


FIG. 24 (a) and (b). A check list and chart of the existing knowledge of the genus *Plasmodium* and its closely related genera.

The genus *Plasmodium* is redefined as a parasitic protozoan which reproduces sexually and by sporogony in an anopheline mosquito and asexually by schizogony in two cycles, one, producing pigment, in non-nucleated red blood cells, and the other in parenchyma cells of the liver, producing considerably more than 1,000 merozoites. The genus *Haemamoeba* is redefined as a parasitic protozoan which *reproduces sexually and by sporogony* in the family Culicidae, and asexually by *schizogony in two cycles, one producing pigment in nucleated red blood cells, the other in endothelial and haemopoietic cells* producing considerably less than 1,000 merozoites.

* *The* International Rules of Zoological Nomenclature allow the alternative generic name *Laverania* for the species *falciparum* and *reichenowi* for the use of those workers who wish to place these two species in a separate genus on the basis of the shape of the gametocytes. Thus, should workers wish to do so on the evidence of the e-e cycle as well, no great nomenclatural upheaval is necessary.

THE PRESENT STATE OF OUR KNOWLEDGE OF THE BIOLOGY OF MA
AND RELATED PARASITES.

FAMILY	GENUS	SPECIES	GAMETOGONY	SPOROGONY	SCHIZOGONY: Pre-erythrocytic	SCHIZOGONY: Erythrocytic	SCHIZOGONY: Exo-erythrocytic	HOST
PLASMODIIDAE	PLASMODIUM	falciparum						MAN
		vivax						
		malariae						
		ovale						
		reichenowi						HIGHER APES
		schwetzi						
		hylobati						
		pitheci						
		cynomolgi						LOWER APES
		inui						
		gonderi						
		simium						
		brasilianum						
		knowlesi						
		girardi						LEMURS
		berghei						RODENTS
		vinckei						
		gallinaceum						BIRDS
		relictum						
		cathemerium						
		lophurae						
		fallax						
		circumflexum						
		juxtanucleare						
		huffi						
		durae						
		nucleophilum						
		elongatum						
		hexamerium						
		polare						
		vaughani						
		rouxi						
		mexicanum						LIZARDS
		floridense						
		rhadinurum						
		minasense						
		tropiduri						
		stenocerci						
		diploglossi						
		carinii						
		lacertiliae						
		pifanoi						
		agamae						
		mabuiae						
		maculilabre						
		giganteum						
		roussetti						BATS
		bufonis						FROGS
		catesbiana						

synonym Pr
synonym P. (
synonym P.ly

FIG. 24 (b).

KNOWN — IMPERFECTLY KNOWN — REPORTED BUT DOUBTFUL — UNKNOWN — DOESN'T OCCUR

The genus *Haemamoeba* Feletti and Grassi, 1889, takes precedence over the genus *Proteosoma* Labbé, 1894, and as Feletti and Grassi designed the genus to include the avian malaria parasites as distinct from both *Haemoproteus* (their *Laverania*) and the mammalian plasmodia their designation is taken as correct. The description of the genus by Feletti and Grassi, in a now lost pamphlet of about the 23rd December 1889 which appeared again in *La Riforma Medica* of 15 January 1890, takes precedence over the later paper on the same subject by Grassi and Feletti later in the year 1890.

A check list of *Plasmodium* and related genera is set out in fig. 24, including the general knowledge of these parasites to date. The above sub-division of the Plasmodiidae has not been included.

BIBLIOGRAPHY

ACTON, H. W., & KNOWLES, R. (1914). Studies on the Halteridium parasite of the pigeon, *Haemoproteus columbae* Celli & San Felice. *Indian J. med. Res.*, **1**, 663–90.

ADLER, S., & TCHERNOMORETZ, I. (1941). Continued passage of extra-erythrocytic forms of *Plasmodium gallinaceum* in the absence of erythrocytic schizogony. *Ann. trop. Med. Parasit.*, **35**, 241–6.

—— (1943). The extra-erythrocytic origin of gametocytes of *Plasmodium gallinaceum* Brumpt, 1935. *Ann. trop. Med. Parasit.*, **37**, 148–51.

ALESSANDRO, G. D', ODDO, F., & SMIRAGLIA, C. (1948). Sulla fase di latenza nell' infezione da *Plasmodium gallinaceum*. *Boll. Soc. ital. Biol. sper.*, **34**, 1036–8.

ALVAREZ, D. A., Jr. (1952). Phagocytosis and destruction of *Plasmodium gallinaceum* by cells of the reticulo-endothelial system. *Amer. J. Hyg.*, **56**, 31–8.

ALVING, A. S. (1948). Pentaquine (S. N. 13,276) and Isopentaquine (S. N. 13,274), therapeutic agents effective in reducing relapse rate in *vivax* malaria. *Proc. 4th int. Congr. trop. Med. Malaria*, Wash., **1**, 734–41.

ALVING, A. S., ARNOLD, J., & ROBINSON, D. H. (1952). Status of Primaquine. 1. Mass therapy of sub-clinical *vivax* malaria with primaquine. *J. Amer. med. Ass.*, **149**, 1558–62.

AMERICANO FREIRE, S. (1946). The influence of sporozoite quantity upon the prophylactic action of sulfadiazine and sulfanilamide on avian malaria, *P. gallinaceum*. *Rev. bras. Biol.*, **6**, 51.

AMERICANO FREIRE, S., & LOBATO PARAENSE, W. (1944). The prophylactic and curative action of sulfadiazine (2-sulfanilamide-pyrimidine), sulfapyridine (2-sulfanilamide-pyridine) and sulfanilamide (p-aminobenzo-sulfonamide) on the erythro and exo-erythrocytic cycle of *Plasmodium gallinaceum*. (Therapeutic and parasitological aspects.) *Rev. bras. Biol.*, **4**, 27.

ANDREWS, W. H. H., GALL, D., & MAEGRAITH, B. G. (1947). Studies on synthetic antimalarial drugs. XIX. The effect of therapeutic courses of Paludrine on the relapse rate of *vivax* malaria. *Ann. trop. Med. Parasit.*, **41**, 375–9.

ANGELINI, G. (1947). Incertezza dei reperti di " forme esoeritrocitiche " dei plasmodi della malaria umana nel midollo osseo. *Riv. Parassit.*, **8**, 5–18.

ANSCHUTZ, G. (1909). Ueber den Entwickelungsgang des *Haemoproteus orizivorae* nov. spec. *Zbl. Bakt.*, **51**, 654–9.

ANSCHUTZ, H. (1910). Ueber Uebertragungsversuche von *Haemoproteus orizivorae* und *Trypanosoma paddae*, nebst Bemerkungen über den Entwickelungsgang des ersteren. *Zbl. Bakt.*, Abt. I., Orig., **54**, 328–31.

ARAGÃO, H. DE B. (1908). Über den Entwickelungsgang und die Übertragung von *Haemoproteus columbae*. *Arch. Protistenk.*, **12**, 154–67.

—— (1911). Observações sobre algumas hemogregarinas das aves. *Mem. Inst. Osw. Cruz*, **3**, 54–64.

ARCHETTI, I. (1941). L'azione dei medicamenti sui parassiti malarici. *Riv. Parassit.*, **5**, 129–31.

ARDIAS, A. (1948). La solfonoterapia della malaria. *Arch. ital. Sci. med. colon.*, **29**, 115–33.

ASCOLI, M., & ALESSANDRO, G. D' (1950). Some remarks on the life cycle of the malaria parasite in the human host. *Acta trop., Basel*, **7**, 367–71.

—— (1951). Sulla genesi delle recidive nell' infezione malarica. *Rif. med.*, **65**, 1213–18.

ASSENDELFT, F. VAN (1931). Impfmalaria. *Beih. Arch. Schiffs- u. TropHyg.*, **1**, 35, 1–104.

Baldi, A. (1952). Sull' infezione da *P. berghei* (Vincke & Lips). *Riv. Malariol.*, 31, 41–52.

Baranger, P., & Filer, M. K. (1951). Action du quinquina sur les formes exo-érythrocytiques de *P. gallinaceum*. *Acta trop., Basel*, 8, 52–9.

—— (1953). De l'action protectrice des colliers dans la malaria aviare. Essai d'ethnographie expérimentale. *Acta trop. Basel.*, 10, 69–72.

Barretto, M. P. (1943). Malária aviária : III. *Sobre o encontro de formas exo-eritrocíticas* do *Plasmodium juxtanucleare* Versiani e Gomes, 1941. (Nota prévia.) *Hospital, Rio de J.*, 24, 643–5.

Becker, E. R., & Manresa, M. (1950). Phanerozoites in turkeys succumbing with blood-induced *Plasmodium lophurae* infection. *Iowa St. Coll. J. Sci.*, 24, 353–4.

Beltran, E. (1944). Protozoarios sanguíneos de las aves. *An. Esc. nac. Cienc. biol., Méx.*, 3, 361–6.

Ben-Harel, S. (1923). Studies of bird malaria in relation to the mechanism of relapse. *Amer. J. Hyg.*, 3, 652–85.

Berghe, L. van den, Vincke, I., & Chardome, M. (1950). La phase tissulaire de *Plasmodium berghei*. *Ann. Soc. belge Méd. trop.*, 30, 79–82.

Berliner, R. W., Earle, D. P., Jr., Taggart, J. V., Welch, W. J., Zubrod, C. G., Knowlton, P., Atchley, J. A., & Shannon, J. A. (1948). Studies on the chemotherapy of the human malarias. VII. The anti-malarial activity of pamaquine. *J. clin. Invest.*, 27, 108-113.

Bianchi, C. (1940). Sul ciclo monogonico primario dei plasmodi malarici nell' uomo. *Ateneo parmense*, 12, 159–68.

Bignami, A. (1891). Ricerche sull' anatomia patologica delle perniciose. *Atti R. Accad. med., Roma*, 16, 2. ser., 5, 291–348.

—— (1913). Concerning the pathogenesis of relapses in malarial fevers. *Sth. med. J. Nashville*, 6, 79–89.

Bishop, A., Birkett, B., & Gilchrist, B. M. (1947). The response of blood-inoculated and sporozoite-induced infections of *Plasmodium relictum* to drugs. *Parasitology*, 38, 163–72.

Bishop, A., & McConnachie, E. W. (1948). Resistance to sulphadiazine and "Paludrine" in the malaria parasite of the fowl (*P. gallinaceum*). *Nature, Lond.*, 162, 541.

Black, R. H. (1947). The consumption of haemoglobin by malaria parasites. *Ann. trop. Med. Parasit.*, 41, 215–17.

Blanc, F., & Cros, R. (1952). Note sur la présence d'un schizonte dans un monocyte du sang circulant. *Méd. trop.*, 12, 83–4.

Blanc, F., & Languillon, J. (1949). A propos des *formes exo-érythrocytiques* de l'hématozoaire de Laveran. *Méd. trop.*, 9, 336–48.

Born, W. C. (1952). Malaria und Diabetes mellitus. *Z. Tropenmed. u. Parasit.*, 3, 503–7.

Boyd, J. S. K. (1953). Treatment of acute malaria. *Brit. med. J.*, *ii*, 392.

Boyd, M. F. (1940). The influence of sporozoite dosage in *vivax* malaria. *Amer. J. trop. Med.*, 20, 279–86.

—— (1947). A review of the studies on immunity to *vivax malaria*. *J. nat. Malar. Soc.*, 6, 12–31.

Boyd, M. F., & Kitchen, S. F. (1939). The demonstration of sporozoites in human tissues. *Amer. J. trop. Med.*, 19, 27–31.

—— (1944). Renewed clinical activity in naturally induced *vivax* malaria. *Amer. J. trop. Med.*, 24, 221–34.

Boyd, M. F., & Matthews, C. B. (1939). An observation on the incubation period of *Plasmodium falciparum*. *Amer. J. trop. Med.*, 19, 69–71.

Boyd, M. F., & Stratman-Thomas, W. K. (1934). Studies in benign tertian malaria. 7. Some observations on inoculation and onset. *Amer. J. Hyg.*, 20, 488–95.

Brackett, S. (1946). Screening tests for antimalarial activity. Code symbol O. In Wiselogle, F. Y. (Ed.), *A Survey of Antimalarial Drugs 1941–45*, Ann Arbor, Mich.: J. W. Edwards. 1, 491–5.

Brackett, S., & Hughes, C. O. (1945). Chilling as a means of retaining the viability of the sporozoites of *Plasmodium gallinaceum*. *J. Parasit.*, 31, 288–9.

Brackett, S., & Waletzky E. (1946a). The antimalarial action of metachloridine (2 metanilamido-5-chloropyrimidine) and other metanilamide derivatives in test infections with *Plasmodium gallinaceum*. *J. Parasit.*, 32, 325–39.

Brackett, S., & Waletzky, E. (1946b). The inability of drugs of both causal prophylactic and suppressive action to cure established infections with avian malaria. *J. Parasit.*, 32 (No. 5, Sect. 2), 8–9.

Brackett, S., Waletzky, E., & Baker, M. (1945). Rate of action of sulfadiazine and quinine on the malarial parasite, *Plasmodium gallinaceum*. *J. Pharmacol.*, 84, 254–61.

—— (1946). The relation between pantothenic acid and *P. gallinaceum* infections in the chicken and the antimalarial activity of analogues of pantothenic acid. *J. Parasit.*, 32, 453–62.

Bray, R. S. (1954). On the coccidia of the mongoose. *Ann. trop. Med. Parasit.*, 48, 405–15.

Bray, R. S., & Garnham, P. C. C. (1953a). Effect of milk diet on *P. cynomolgi* infections in monkeys. *Brit. med. J.*, i, 1200.

—— (1953b). Histo-chemical reactions of the exo-erythrocytic cycle in malaria. [Demonstration.] *Trans. R. Soc. trop. Med. Hyg.*, 47, 263.

Bray, R. S., & Williamson, J. (1953). The development of the pre-erythrocytic cycle of *Plasmodium cynomolgi* in normal and glycogen-depleted monkey livers. [Demonstration.] *Trans. R. Soc. trop. Med. Hyg.*, 47, 263–4.

Brug, S. L. (1940). Exo-erythrozytäre Malariaparasiten beim Menschen. *Riv. Malariol.*, 19 (sez. I), 226–9.

—— (1941). Nachtrag zu meiner Arbeit "Exo-erythrozytäre Malariaparasiten beim Menschen". *Riv. Malariol.*, 20 (sez. I), 66–7.

Brumpt, E. (1937). Schizogonie parfois intense du *Plasmodium gallinaceum* dans les cellules endothéliales des poules. *C. R. Soc. Biol., Paris*, 125, 810–13.

Brumpt, E., Bovet, D., & Brumpt, L. (1937). Action des médicaments anti-paludiques sur l'infection de la poule par le *Plasmodium gallinaceum*. *Festschr. B. Nocht*, 61–6.

Buck, A. de (1936). Some results of six years' mosquito infection work. *Amer. J. Hyg.*, 24, 1–18.

Carini, A., & Maciel, J. (1916). Quelques hémoparasites du Brésil. *Bull. Soc. Pat. exot.*, 9, 247–65.

Carpano, M. (1942). Sulle due fasi endo- ed eso-eritrocitarie del ciclo evolutivo della *Theileria parva* (Theiler, 1904), e del *Plasmodium gallinaceum* Brumpt, 1935 nell' ospite vertebrato. *Riv. Parassit.*, 6, 187–211.

Carrington, H. C., Crowther, A. F., Davey, D. G., Levi, A. A., & Rose, F. L. (1951). A metabolite of "Paludrine" with high antimalarial activity. *Nature, Lond.*, 168, 1080.

Casini, G. (1939). La fase apigmentata di evoluzione dei plasmodidi nella malaria cronica. *Riv. Malariol.*, 18 (sez. I), 73–5.

Cavaceppi, L. (1951). Durata del ciclo endoistiocitario del *Plasmodium gallinaceum* determinata mediante ripetute biopsie di corteccia cerebrale. *Riv. Parassit.*, 12, 163–8.

Chen, T. T. (1944). The nuclei in avian malaria parasites. I. The structure of nuclei in *Plasmodium elongatum* with some considerations on technique. *Amer. J. Hyg.*, 40, 26–34.

Chorris, P. (1938a). Su alcuni stadi di sviluppo del *Plasmodium gallinaceum* Brumpt, 1935. 1. nota. *Riv. Parassit.*, 2, 121–8.

—— (1938b). Su alcuni stadi di sviluppo del *Plasmodium gallinaceum* Brumpt. *R. C. Ist. Sanit. pubbl.*, 1, 532–9.

CHORTIS, P. (1938c). *Sulle alterazioni del sistema reticulo-endoteliale nella infezioni da Plasmodium gallinaceum. Riv. Parassit.*, 2, 315–22.

CIUCA, M., BALLIF, L., CHELARESCU, M., ISANOS, M., & GLASER, L. (1937a). Action of quinine and atebrin on the sporozoites of *P. falciparum. Trans. R. Soc. trop. Med. Hyg.*, 31, 235–40.

—— (1937b). On drug prophylaxis in therapeutic malaria. *Trans. R. Soc. trop. Med. Hyg.*, 31, 241–4.

—— (1937c). Contributions à l'étude de la tierce maligne expérimentale. Pourvoir infectant du sang au cours de l'incubation. *Riv. Malariol.*, 16 (sez. 1), 85–90.

CLARKE, D. H. (1952). The use of phosphorus 32 in studies on *Plasmodium gallinaceum*. I. The development of a method for the quantitative determination of parasite growth and development *in vitro. J. exp. Med.*, 96, 439–50.

CLARKE, D. H., & THEILER, M. (1948). Studies on parasite-host interplay between *Plasmodium gallinaceum* and the chicken as influenced by hydroxynaptha-quinones. *J. infect. Dis.*, 82, 138–62.

COATNEY, G. R. (1933). Relapse and associated phenomena in the *Haemoproteus* infections of the pigeon. *Amer. J. Hyg.*, 18, 133–60.

—— (1952). Studies on the compound 50–63. *Trans. R. Soc. trop. Med. Hyg.*, 46, 496–7.

COATNEY, G. R., CLARK, W. C., YOUNG, M. D., & MCLENDON, S. B. (1947). Studies in human malaria. I. The protective action of sulfadiazine and sulfapyrazine against sporozoite-induced *falciparum* malaria. *Amer. J. Hyg.*, 46, 84–104.

COATNEY, G. R., & COOPER, W. C. (1944). The prophylactic effect of sulfadiazine and sulfaguanidine against mosquito-borne *Plasmodium gallinaceum* infection in the domestic fowl. (Preliminary report.) *Publ. Hlth Rep., Wash.*, 59 (ii), 1455–8.

—— (1948a). A symposium on exo-erythrocytic forms of malarial parasites. III. The chemotherapy of malaria in relation to our knowledge of exo-erythrocytic forms. *J. Parasit.*, 34, 275–89.

—— (1948b). Recrudescence and relapse in *vivax* malaria. *Proc. 4th int. Congr. trop. Med. Malaria*, 1, 629–39.

COATNEY, G. R., COOPER, W. C., EYLES, D. E., CULWELL, W. B., WHITE, W. C., & LINTS, H. A. (1950). Studies in human malaria. XXVII. Observations on the use of Pentaquine in the prevention and treatment of Chesson strain *vivax* malaria. *J. nat. Malar. Soc.*, 9, 222–33.

COATNEY, G. R., COOPER, W. C., & MILES, V. I. (1945). Studies on *Plasmodium gallinaceum* Brumpt. I. The incidence and course of the infection in young chicks resulting from single mosquito bites. *Amer. J. Hyg.*, 41, 109–18.

COATNEY, G. R., COOPER, W. C., RUHE, D. S., YOUNG, M. D., & BURGESS, R. W. (1950). Studies in human malaria. XVIII. The life pattern of sporozoite-induced St. Elizabeth strain *vivax* malaria. *Amer. J. Hyg.*, 51, 200–15.

COATNEY, G. R., COOPER, W. C., & TREMBLEY, H. L. (1945a). Studies on *Plasmodium gallinaceum* Brumpt. II. The incidence and course of the infection in young chicks following the inoculation of infected salivary glands. *Amer. J. Hyg.*, 41, 119–22.

—— (1945b). Studies on *Plasmodium gallinaceum* Brumpt. III. The incidence and course of the infection in young chicks following the subcutaneous inoculation of pooled sporozoites. *Amer. J. Hyg.*, 42, 323–9.

COATNEY, G. R., COOPER, W. C., WHITE, W. C., LINTS, H. A., CULWELL, W. B., & EYLES, D. E. (1950). Studies in human malaria. XXIV. Protective and therapeutic trials of SN 10,751 (camoquin) against the Chesson strain of *Plasmodium vivax. J. nat. Malar. Soc.*, 9, 67–74.

Coatney, G. R., Cooper, W. C., & Young, M. D. (1950). Studies in human malaria. XXX A summary of 204 sporozoite-induced infections with the Chesson strain of *Plasmodium vivax*. *J. nat. Malar. Soc.*, 9, 381–96.

Coatney, G. R., Greenberg, J., Cooper, W. C., & Trembley, H. L. (1949). Antimalarial activity of aureomycin against *Plasmodium gallinaceum* in the chick. *Proc. Soc. exp. Biol., N.Y.*, 72, 586–7.

Coatney, G. R., Myatt, A. V., Hernandez, T., Jeffery, G. M., & Cooper, W. C. (1953). Studies in human malaria. XXXII. The protective and therapeutic effects of pyrimethamine (Daraprim) against Chesson strain *vivax* malaria. *Amer. J. trop. Med. Hyg.*, 2, 777–87.

Coatney, G. R., & Sebrell, W. H. (1946). Screening tests for antimalarial activity and toxicity. Code symbol A. In : Wiselogle, F. Y. (Ed.) *A Survey of Antimalarial Drugs 1941–1945*, Ann Arbor, Mich. : J. W. Edwards, 1, 457–62.

Coggeshall, L. T., & Porter, R. J. (1946). Screening tests for antimalarial activity. Code symbol B. In : Wiselogle, F. Y. (Ed.) *A Survey of Antimalarial Drugs 1941–45*, Ann Arbor, Mich. . J. W. Edwards, 1, 463–6.

Coggeshall, L. T., Porter, R. J., & Laird, R. L. (1944) Prophylactic and curative effects of certain sulfonamide compounds on exo-erythrocytic stages in *Plasmodium gallinaceum* malaria. *Proc. Soc. exp. Biol., N.Y.*, 57, 286–92.

Coggeshall, L. T., & Rice, F. A. (1949). Cure of chronic *vivax* malaria with pentaquin. *J. Amer. med. Ass.*, 139, 437–8.

Coles, A. C. (1914). Blood parasites found in mammals, birds and fishes in England. *Parasitology*, 7, 17–61.

Conti, F., & Monaco, R. (1950). Fase pre-eritrocitica e fase eso-eritrocitica del parassita malarico nell' uomo, al lume di recenti ricerche. *Rif. med.*, 64, 548–51.

—— (1951). Ricerche sulla patogenesi delle recidive malariche. *Rif. med.*, 65, 973–9.

Cooper, W. C. (1949). Summary of antimalarial drugs. *Publ. Hlth Rep., Wash.*, 64, 717–32.

Cooper, W. C., Coatney, G. R., & Imboden, C. A., Jr. (1950). Studies in human malaria. XXIII. Acquired resistance to chlorguanide in the Chesson strain of *P. vivax*. *J. nat. Malar. Soc.*, 9, 59–66.

Cooper, W. C., Coatney, G. R., Jeffery, G. M., & Imboden, C. A., Jr. (1950). Studies in human malaria. XXVIII. Observations on the use of chlorguanide against the Chesson strain of *P. vivax*. *J. nat. Malar. Soc.*, 9, 366–76.

Corradetti, A. (1938a). Alcune osservazioni sul ciclo schizogonico del *Plasmodium gallinaceum* e del *Plasmodium cathemerium*. *Riv. Malariol.*, 17, 15–19.

—— (1938b). Su alcune fasi del ciclo schizogonico del *P. gallinaceum* e del *P. cathemerium*. *R. C. Accad. Lincei, Cl. sci. fis. mat. nat.*, 27, 121–2.

—— (1938c). Tentativo di ricostruzione del ciclo generale dei plasmodi nell' ospite vertebrato. *R. C. Accad. Lincei, Cl. sci. fis. mat. nat.*, 28, 164.

—— (1938d). Osservazioni sul ciclo schizogonico dei plasmodi nelle cellule dei tessuti e proposta di una nuova classificazione degli Haemosporidiidea. *Riv. Parassit.*, 2, 23–37.

—— (1938e). Una nuova classificazione degli Haemosporidiidea basata sull' esistenza di un ciclo schizogonico dei plasmodi nelle cellule dei tessuti. *R. C. Accad. Lincei, Cl. sci. fis. mat. nat.*, 27, 31–2.

—— (1938f). Sullo sviluppo dei parassiti malarici. *Riv. Malariol.*, sez. II, 17, 363–5.

—— (1940a). Ricerche sulla biologia del *Plasmodium gallinaceum* nei polli inoculati con sangue infetto. *Riv. Parassit.*, 4, 249–76.

—— (1940b). Recensione. *Riv. Parassit.*, 4, 280.

—— (1940c). Studi sul *Plasmodium gallinaceum*. I. Sulla produzione del ciclo eso-eritrocitico negli animali inoculati con sangue infetto. *Boll. Soc. ital. Biol. sper.*, 15, 678–80.

Corradetti, A. (1940d). Studi sul *P. gallinaceum*. II. Dimostrazione della non attendibilità della teoria che il ciclo eso-eritrocitico derivi esclusivamente dagli sporozoiti o da precedenti forme eso-eritrocitiche. *Boll. Soc. ital. Biol. sper.*, 15, 680–1.

—— (1940e). Inesistenza di un ciclo schizogonico del *P. elongatum* in cellule dell' apparato reticolo-endoteliale. *Boll. Soc. ital. Biol. sper.*, 15, 825.

—— (1940f). Il comportamento dei macrofagi nelle infezioni da *P. elongatum*. *R. C. Ist. San. pubbl.*, 3, 647–52.

—— (1940g). The significance of the exo-erythrocytic cycle of Plasmodia. *J. trop. Med. Hyg.*, 43, 110–13.

—— (1940h). Recensione. *Riv. Parassit.*, 4, 283.

—— (1940i). Nuovi studi sui plasmodi della malaria. *Policlinico, sez. prat.*, 47, 546–8.

—— (1940j). Studi sul *P. gallinaceum*. III. Sui rapporti tra ciclo eso-eritrocitico e recidive. *Boll. Soc. ital. Biol. sper.*, 15, 995–6.

—— (1941a). Valore biologico del ciclo endoistiocitario del *Plasmodium gallinaceum*. *Riv. Parassit.*, 5, 209–16.

—— (1941b). Ricerche sulla patologia dell' infezione da *Plasmodium gallinaceum* nei polli inoculati con sangue infetto. *Riv. Parassit.*, 5, 141–54.

—— (1941c). Studi sul *P. gallinaceum*. V. Osservazioni sulla fagocitosi nel midollo osseo di polli inoculati con sangue infetto. *Boll. Soc. ital. Biol. sper.*, 16, 135–6.

—— (1941d). Die neuen Theorien über die Pathologie der Malaria im Lichte unserer Kenntnisse von der vergleichenden Biologie der Plasmodien. *Dtsch. tropenmed. Z.*, 45, 591–8.

—— (1941e). Recensione. *Riv. Parassit.*, 5, 133–9.

—— (1941f). Recensione. *Riv. Parassit.*, 5, 139.

—— (1941g). Sul comportamento dei vari ceppi di *P. praecox*. *Boll. Soc. ital. Biol. sper.*, 16, 671.

—— (1941h). Sul comportamento di un ceppo di *P. cathemerium*. *Boll. Soc. ital. Biol. sper.*, 16, 670.

—— (1942a). Il ciclo schizogonico degli emosporidi nell' ospite vertebrato. *Riv. Biol.*, 33, 113–53.

—— (1942b). Osservazioni sulla biologia del *P. gallinaceum* nel tacchino. *Riv. Parassit.*, 6, 43–8.

—— (1942c). Die biologische Bedeutung des endohistiozytären Zyklus des *P. gallinaceum*. *Zbl. Bakt.*, 1. Abt., *Orig.*, 148, 274–9.

—— (1943). Der schizogone Zyklus der Hämosporidien im Wirbeltier. *Arch. Protistenk.*, 96, 235–87.

—— (1949). Studi sulla patologia e immunologia comparate nelle infezioni da emosporidi. *Riv. Parassit.*, 10, 129–41.

—— (1952). Études de pathologie et d'immunologie comparées dans le paludisme de l'homme et des animaux. *Riv. Malariol.*, 31, 127–34.

Corradetti, A., & Cavaceppi, L. (1952). Relations between the endohistiocytic forms of *P. gallinaceum* and the immunological course of the infection in fowls inoculated with infected brain tissue. *Amer. J. trop. Med. Hyg.*, 1, 761–7.

Corradetti, A., & Gramiccia, G. (1941a). Ricerche sul valore biologico del ciclo schizogonico del *P. elongatum* nelle cellule ematopoietiche. *Riv. Parassit.*, 5, 5–16.

—— (1941b). Decorso comparativo dell' infezione da *Plasmodium elongatum* nel sangue e nelle cellule ematopoietiche. *Boll. Soc. ital. Biol. sper.*, 16, 133–5.

—— (1943). Azione dei medicamenti sulle forme del ciclo schizogonico del *P. elongatum* in relazione con il tipo di cellula ospite. *Riv. Parassit.*, 7, 99–110.

Corradetti, A., & Verolini, F. (1950). Studies on relapses in blood-induced infections from *P. malariae* and *P. cynomolgi*. *J. nat. Malar. Soc.*, 9, 327–31.

Corradetti, A., & Verolini, F. (1951). Studi sulle recidive da *P. malariae* e da *P. cynomolgi* in infezioni indotte con sangue. *R. C. Ist. sup. Sanit.*, 14, 271–81.
Coudert, J. (1945). Le stade réticulo-endothélial du paludisme de l'homme. *Rev. Palud. Méd. trop.*, 3, 62–3.
Coudert, J., & Eyquem, A. (1944). Le stade réticulo-endothélial du paludisme de l'homme. *J. méd. Lyon*, 25, 305–9.
Coulston, F. (1940). A practicable semi-permeable capsule suitable for avian malaria studies. *J. Parasit.*, 26, suppl. 30.
—— (1949). Exo-erythrocytic stages of *P. cynomolgi* in the *Macaca mulatta*. *Proc. Soc. exp. Biol., N.Y.*, 70, 360–4.
Coulston, F., Cantrell, W., & Huff, C. G. (1945). The distribution and localisation of sporozoites and pre-erythrocytic stages in infections with *P. gallinaceum*. *J. infect. Dis.*, 76, 226–38.
Coulston, F., & Huff, C. G. (1947). The morphology of cryptozoites and metacryptozoites of *P. relictum* and the relationship of these stages to parasitaemia in canaries and pigeons. *J. infect. Dis.*, 80, 209–17.
—— (1948). Symposium on exo-erythrocytic forms of malarial parasites. IV. The chemotherapy and immunology of pre-erythrocytic stages in avian malaria. *J. Parasit.*, 34, 290–9.
Coulston, F., & Manwell, R. D. (1941). Single parasite infections and exo-erythrocytic schizogony in *P. circumflexum*. *Amer. J. Hyg.*, 34, sect. C, 119–25.
Coulston, F., & Robinson, F. O. (1950). Artefacts and exo-erythrocytic stages of *P. cynomolgi* in *Macaca mulatta*. *J. Parasit.*, 36, 28–9.
Covell, G., Russell, P. F., & Swellengrebel, N. H. (1953). *Malaria terminology*: report of a drafting committee. W. H. O. *Monog. Ser. No. 13. Geneva.*
Covell, G., Shute, P. G., & Maryon, M. (1953a). Pyrimethamine (Daraprim) as a prophylactic agent against a West African strain of *P. falciparum*. *Brit. med. J.*, i, 1081–3.
—— (1953b). Pyrimethamine (Daraprim) in the treatment of *vivax* malaria. *Brit. med. J.*, ii, 258–9.
Craig, C. F. (1910). Studies in the morphology of malarial plasmodia after the administration of quinine and in intracorpuscular conjugation. *J. infect. Dis.*, 7, 285–318.
Craige, B., Jr., Alving, A. S., Jones, R., Whorton, C. M., Pullman, T. N., & Eichelberger, L. (1947). The Chesson strain of *P. vivax* malaria. II. Relationship between prepatent period, latent period, and relapse rate. *J. infect. Dis.*, 80, 228–236.
Curd, F. H. S. (1943). The activity of drugs in the malaria of man, monkeys, and birds. *Ann. trop. Med. Parasit.*, 37, 115–43.
Curd, F. H. S., Davey, D. G., & Rose, F. L. (1945). Studies on synthetic anti-malarial drugs. X. Some biguanide derivatives as new types of antimalarial substances with both therapeutic and causal prophylactic activity. *Ann. trop. Med. Parasit.*, 39, 208–16.
Danielli, J. F. (1946). A critical study of techniques for determining the cytological position of alkaline phosphatase. *J. exp. Biol.*, 22, 110–17.
Danilewsky, V. (1890). Développement des parasites malariques dans les leucocytes des oiseaux. *Ann. Inst. Pasteur*, 4, 427–31.
Davey, D. G. (1944). Biology of the malarial parasite in the vertebrate host. *Nature, Lond.*, 153, 110–11.
—— (1946a). Concerning exo-erythrocytic forms and the evidence for their existence in human malaria. *Trans. R. Soc. trop. Med. Hyg.*, 40, 171–82.
—— (1946b). The use of avian malaria for the discovery of drugs effective in the treatment and prevention of human malaria. II. Drugs for causal prophylaxis and radical cure or the chemotherapy of exo-erythrocytic forms. *Ann. trop. Med. Parasit.*, 40, 453–71.

DEARBORN, E. H., & MARSHALL, E. K., Jr. (1947). The susceptibility of different species of avian malarial parasites to drugs. *Amer. J. Hyg.*, 45, 25–8.

DECOURT, PH. (1949a). Les stades exo-érythrocytaires du *Plasmodium* : la forme pré-schizontique (protozoite) et la phase post-schizontique. *Leurs rapports avec l'immunité. Bull. Soc. Pat. exot.*, 42, 452–6.

—— (1949b). Discussion. *Bull. Soc. Pat. exot.*, 42, 263–7.

DECOURT, P., & SCHNEIDER, J. (1938a). Les lacunes de nos connaissances sur le cycle plasmodial chez l'hôte vertébré. *Bull. Soc. Pat. exot.*, 31, 603–9.

—— (1938b). Note préliminaire sur la recherche de la localisation et de la morphologie des plasmodes pendant les périodes d'infestation latente. *Bull. Soc. Pat. exot.*, 31, 609–14.

DOBLER, M. (1941). Exoerythrocytic schizogony associated with the matinal strain of *P. relictum* after passage through ducks. *Amer. J. Hyg.*, 34, sect. C, 49.

DOWNS, W. G. (1947). Infections of chicks with single parasites of *P. gallinaceum* Brumpt. *Amer. J. Hyg.*, 46, 41–4.

DRAPER, C. C. (1953). Observations on the reciprocal immunity between some avian plasmodia. *Parasitology*, 43, 139–42.

DUBIN, I. N. (1947). Bodies suggesting exo-erythrocytic forms of *P. vivax* in tissue culture. *Proc. Soc. exp. Biol., N.Y.*, 65, 154–6.

—— (1948). A search for exo-erythrocytic forms of human malaria by means of tissue cultures of bone marrow. *J. nat. Malar. Soc.*, 7, 330–2.

—— (1950). Effect of variations in osmotic pressure on macrophages in tissue culture. *Proc. Soc. exp. Biol., N.Y.*, 75, 250–2.

—— (1952). The cultivation of the exoerythrocytic forms of *P. gallinaceum* in tissue culture. *J. infect. Dis.*, 91, 33–49.

DUBIN, I. N., LAIRD, R. L., & DRINNON, V. P. (1949). The development of sporozoites of *P. gallinaceum* into cryptozoites in tissue culture. *J. nat. Malar. Soc.*, 8, 175–80.

—— (1950). Further observations on the development of sporozoites of P. *gallinaceum* into cryptozoites in tissue culture. *J. nat. Malar. Soc.*, 9, 119–27.

DUBIN, I. N., & YEN, C. K. (1950). Range of extracellular hydrogen ion concentration tolerated by macrophages grown in tissue culture. *Arch. Path.*, 50, 562–77.

EDESON, J. F. B. (1953). Presumed exo-erythrocytic schizonts of *P. knowlesi* in the liver of a Malayan monkey (*Macaca irus*). *Trans. R. Soc. trop. Med. Hyg.*, 47, 399–400.

EDESON, J. F. B., & FIELD, J. W. (1951). *Hepatocystis vassali* in the Malayan squirrel. *Trans. R. Soc. trop. Med. Hyg.*, 44, 356.

EDGCOMB, J. H., ARNOLD, J., YOUNG, E. H., Jr., ALVING, A. S., EICHELBERGER, L., JEFFERY, G. M., EYLES, D., & YOUNG, M. D. (1950). Primaquine SN 13272, a new curative agent in *vivax* malaria ; a preliminary report. *J. nat. Malar. Soc.*, 9, 285–92.

FABIANI, G., VARGUES, R., FULCHIRON, G., GRELLET, P., & VERAIN, A. (1951). Facteurs et signification de la période prépatente dans le paludisme expérimental à *P. berghei*. *Bull. Soc. Pat. exot.*, 44, 580–91.

FAIRLEY, N. H., *et al.* (1946a). Researches on "Paludrine" (M4888) in malaria ; an experimental investigation undertaken by the L.H.Q. medical research unit (A.I.F.), Cairns, Australia. *Trans. R. Soc. trop. Med. Hyg.*, 40, 105–51.

—— (1946b). Researches on "Paludrine" (M4888) in Australia. *Med. J. Aust.*, 33 (i), 234–6.

—— (1947). Sidelights on malaria in man obtained by sub-inoculation experiments. *Trans. R. Soc. trop. Med. Hyg.*, 40, 621–76.

FAIRLEY, N. H. (1949). Malaria, with special reference to certain experimental, clinical and chemotherapeutic investigations. *Brit. med. J.*, ii, 825–30.

FAIRLEY, N. H. (1952). The chemoprophylaxis and chemotherapy of malaria in man with special reference to the life cycle. *Australas. Ann. Med.*, 1, 7–17.

FALCO, E. A., GOODWIN, L. G., HITCHINGS, G. H., ROLLO, I. M., & RUSSELL, P. B. (1951). 2-4-Diaminopyrimidines—a new series of anti-malarials. *Brit. J. Pharmacol.*, 6, 185–200.

FALCO, E. A., HITCHINGS, G. H., RUSSELL, P. B., & VAN DER WERFF, H. (1949). Anti-malarials as antagonists of purines and pteroylglutamic acid. *Nature, Lond.*, 164, 170–8.

FANTHAM, H. B. (1910a). Observations on the parasitic protozoa of the red grouse (*Lagopus scoticus*), with a note on the grouse fly. *Proc. zool. Soc. Lond.*, ii, 692–708.

—— (1910b). On the occurrence of schizogony in an avian leucocytozoön, *L. lovati*, parasitic in the red grouse, *Lagopus scoticus*. *Ann. trop. Med. Parasit.*, 4, 255–60.

FANTHAM, H. B., & PORTER, A. (1944). On a *Plasmodium* (*P. relictum* var. *spheniscidae* n. var.) observed in four species of penguins. *Proc. zool. Soc. Lond.*, 114, 279–92.

FELL, H. B., & ROBISON, R. (1929). The growth, development and phosphatase activity of embryonic avian femora and limb-buds cultivated *in vitro*. *Biochem. J.*, 23, 767–84.

FIELD, J. W. (1947). Paludrine: a general review. *Med. No. 3 of 1947. Malaria Adv. Board, Malayan Union.* Kuala Lumpur.

FIELD, J. W., & EDESON, J. F. B. (1949a). A malaria parasite of the Malayan squirrel. *Bull. Inst. med. Res. F.M.S.*, 2.

—— (1949b). A note on presumed exo-erythrocytic development of *P. vassali* in the liver of the Malayan squirrel. *Trans. R. Soc. trop. Med. Hyg.*, 42, 569–72.

FILIPPINI, A. (1939). Nuove concezioni sulla patologia della malaria. *Policlinico, sez. prat.*, 46, 2054–8.

FINDLAY, G. M. (1951). *Recent Advances in Chemotherapy.* 3rd edit. London: J. A. Churchill Ltd., 2.

FONSECA, F. (1944). Formas exo-eritrocíticas da malária. *Arch. Pat., Lisboa*, 16, 434–66.

FONSECA, F., CAMBOURNAC, F. J. C., PINTO, M. R., PEREIRA, J. M., & CUNHA, A. (1946). Studies on the exo-erythrocytic cycle of malaria. *Parasitology*, 37, 113–17.

FRANCHINI, G. (1923). Hématozoaires de quelques oiseaux d'Italie. *Bull. Soc. Pat. exot.*, 16, 118–25.

FRÓES, H. P. (1938). Uma tentativa de interpretação científica das formas "duplas", "triplas" e "dobradas" da malaria. *Riv. Malariol.*, sez. I, 17, 154–61.

FULTON, J. D. (1939). Experiments on the utilization of sugars by malarial parasites (*P. knowlesi*). *Ann. trop. Med. Parasit.*, 33, 217–27.

GALLAIS, P., CROS, R., & CAPPONI, M. (1949). Les stades exo-érythrocytiques du paludisme humain et la moelle osseuse étudiée en frottis. Analyse de 104 prélèvements. *Méd. trop.*, 9, 307–35.

GALLIARD, H., & LAPIERRE, J. (1950). Récherches sur le développement initial de *P. berghei* *Bull. Soc. Pat. exot.*, 43, 410–13.

GARNHAM, P. C. C. (1947). Exo-erythrocytic schizogony in *P. kochi* Laveran. A preliminary note. *Trans. R. Soc. trop. Med. Hyg.*, 40, 719–22.

—— (1948a). The development cycle of *Hepatocystis* (*Plasmodium*) *kochi* in the monkey host. *Trans. R. Soc. trop. Med. Hyg.*, 41, 601–16.

—— (1948b). Exo-erythrocytic schizogony in malaria. *Trop. Dis. Bull.*, 45, 831–44.

—— (1950a). Blood parasites of East African vertebrates, with a brief description of exo-erythrocytic schizogony in *P. pitmani*. *Parasitology*, 40, 328–37.

—— (1950b). Exo-erythrocytic schizogony in bat malaria. *Nature, Lond.*, 166, 155.

—— (1951a). Patterns of exoerythrocytic schizogony. *Brit. med. Bull.*, 8, 10–15.

—— (1951b). The mosquito transmission of *P. inui* Halberstedter and Prowazek, and its pre-erythrocytic development in the liver of the rhesus monkey. *Trans. R. Soc. trop. Med. Hyg.*, 45, 45–52.

Garnham, P. C. C. (1951c). An attempt to find the vector of *Hepatocystis* (=*Plasmodium*) *kochi* Levaditi and Schoen. *Exp. Parasit.*, 1, 94–107.

—— (1951d). Resemblances between "exo-erythrocytic" parasites of *Plasmodium elongatum* in the greenfinch and *P. berghei* in the rat. *Trans. R. Soc. trop. Med. Hyg.*, 45, 2.

—— (1951e). Bat malaria. *Hepatocystis epomophori* in East African fruit bats. *Trans. R. Soc. trop. Med. Hyg.*, 44, 357.

—— (1953a). Terminology of Haemosporidiidea. *5. Congr. int. Méd. trop. Palud.*, Istanbul, *Communications*, 2, 228–31.

—— (1953b). Types of bat malaria. *Riv. Malariol.*, 32, 149–54.

—— (1954). The life history of the malaria parasite. *Lectures on the Scientific Basis of Medicine*, 1952–53 (*Brit. Postgrad. Med. Fed. Univ. Lond.*), 2, 323–33.

Garnham, P. C. C., Bray, R. S., Cooper, W., Lainson, R., Awad, F. I., & Williamson, J. (1954a). Pre-erythrocytic stages of human malaria: *Plasmodium ovale*. A preliminary note. *Brit. med. J.*, i, 257.

—— (1954b). A demonstration. *Trans. R. Soc. trop. Med. Hyg.*, 48, 279.

—— (1955). The pre-erythrocytic stage of *Plasmodium ovale*. *Trans. R. Soc. trop. Med. Hyg.*, 49, 158–67.

Garnham, P. C. C., & Duke, B. O. L. (1953) Certain parasitic protozoa from the Gambia. *Trans. R. Soc. trop. Med. Hyg.*, 47, 7–8.

Garnham, P. C. C., & Heisch, R. B. (1952). A malaria parasite of insectivorous bats, showing exo-erythrocytic schizogony of the *cynomolgi* type. *Trans. R. Soc. trop. Med. Hyg.*, 46, 372–3.

Garnham, P. C. C., & Pick, F. (1952). Unusual form of merocysts of *Hepatocystis* (=*Plasmodium*) *kochi*. *Trans. R. Soc. trop. Med. Hyg.*, 46, 535–7.

Garrison, P. L., Coker, W. G., Jastremski, B., Coatney, G. R., Alving, A. S., & Jones, R., Jr. (1952). Status of Primaquine. 2. Cure of Korean *vivax* malaria with pamaquine and primaquine. *J. Amer. med. Ass.*, 149, 1562–3.

Gavrilov, V., Bobkoff, G., & Laurencin, S. (1938). Essai de culture en tissus de *Plasmodium gallinaceum* (Brumpt). *Ann. Soc. belge Méd. trop.*, 18, 429–34.

Geiling, E. M. K., & Taliaferro, W. H. (1946). Screening tests for antimalarial activity. Code symbol Q. In: Wiselogle, F. Y. (Ed.) *A survey of antimalarial drugs 1941–1945*, Ann Arbor, Mich.: J. W. Edwards, 1, 496–500.

Gill, C. A. (1938). *The seasonal periodicity of malaria*. London: J. & A. Churchill.

Gingrich, W. (1946). Screening tests for antimalarial activity. Code symbol S. In: Wiselogle, F. Y. (Ed.) *A survey of antimalarial drugs 1941–1945*, Ann Arbor, Mich.: J. W. Edwards, 1, 503–4.

Gingrich, W., Schoch, W. W., Schwab, M., & Shepherd, C. C. (1947). Radical cure of avian malaria (*P. cathemerium*) with SN8557, a napthaquinone derivative. *Amer. J. trop. Med.*, 27, 147–52.

Gingrich, W., & Taylor, C. A. (1946). Therapeutic, prophylactic and curative tests in avian malaria (*P. cathemerium*) with metachloridine. *J. Parasit.*, 32, No. 5, Sect. 2 (suppl.), 9.

Giovannola, A. (1938a). Il *Plasmodium gallinaceum* Brumpt, 1935, i così detti corpi *Toxoplasma*-simili ed alcune inclusioni di probabile natura parassitaria nei globuli bianchi del *Gallus gallus*. *Riv. Parassit.*, 2, 139–42.

—— (1938b). Il *Plasmodium gallinaceum* Brumpt, i corpi *Toxoplasma*-simili ed alcune inclusioni di probabile natura parassitaria nei globuli bianchi del *Gallus gallus*. *R. C. Ist. Sanit. pubbl.*, 1, 518–31.

—— (1939). I plasmodi aviari. *Riv. Parassit.*, 3, 221–66.

Goldstein, F. (1939). M. A. Thesis. Syracuse University.

GOLGI, C. (1893). Sulle febbri malariche estivo-autumnali di Roma. *Gazz. med., Pavia*, ii, 481–2; 529–44.

GOMORI, G. (1939). Microtechnical demonstration of phosphatase in tissue sections. *Proc. Soc. exp. Biol. N.Y.*, 42, 23–6.

GOOD, C. A., KRAMER, H., & SOMOGYI, M. (1933). The determination of glycogen. *J. biol. Chem.*, 100, 485–91.

GOODWIN, L. G. (1952). Daraprim—clinical trials and pharmacology. *Trans. R. Soc. trop. Med. Hyg.*, 46, 485–95.

GORDON, R. M., & HANCOX, N. M. (1947). Smears and sections showing the primary phase of *P. gallinaceum* at the site of inoculation with sporozoites. *Trans. R. Soc. trop. Med. Hyg.*, 40, 369–70.

GRAMICCIA, G. (1948). Sulle recenti scoperte relative ad alcune forme di sviluppo degli sporozoiti di *Plasmodium cynomolgi* e di *P. vivax*. *Igiene San. pubbl.*, 4, 74–9.

GRAMICCIA, G., & BLACK, R. H. (1948). The cultivation of exoerythrocytic forms of *P. gallinaceum*. I. A preliminary note. *Ann. trop. Med. Parasit.*, 42, 88–9.

GRAMICCIA, G., & SACCÀ, G. (1947). Note sulla infezione da *P. gallinaceum* nel pulcino. Considerazioni sul significato biologico del ciclo endoistiocitario dopo inoculazione di sangue. *Riv. Parassit.*, 8, 213–19.

GRASSI, B. (1900). Studi di uno zoologo sulla malaria. *R. C. Accad. Lincei.*, 296, Ser. 5a, iii, 289–497.

GREENBERG, J., BOYD, B. L., & JOSEPHSON, E. S. (1948). Synergistic effect of chlorguanide and sulphadiazine against *P. gallinaceum* in the chick. *J. Pharmacol.* 94, 60–4.

GREENBERG, J., COATNEY, G. R., & TREMBLEY, H. L. (1953) The effect of pyrimethamine (Daraprim) against *P. gallinaceum* infections in chicks. *Amer. J. trop. Med. Hyg.*, 2, 771–6.

GREENBERG, J., TREMBLEY, H. L., & COATNEY, G. R. (1950a). Strain differences in *P. gallinaceum* Brumpt. 1. Differences in the behavior of the exo-erythrocytic forms of a blood passaged (BI) and sporozoite passaged (SP) strain of *P. gallinaceum*. *J. nat. Malar. Soc.*, 9, 320–6.

—— (1950b). Effects of drugs on *P. gallinaceum* infections produced by decreasing concentrations of a sporozoite inoculum. *Amer. J. Hyg.*, 51, 194–9.

GRIFFITHS, R. B., & GORDON, R. M. (1952). An apparatus which enables the process of feeding by mosquitoes to be observed in the tissues of a live rodent; together with an account of the ejection of saliva and its significance in malaria. *Ann. trop. Med. Parasit.*, 46, 311–19.

GRIGNASCHI, V. J. (1952). Observaciones sobre la biologia del *Plasmodium gallinaceum* Brumpt 1935. *Riv. Parassit.*, 13, 261–4.

HAAS, V. H., WILCOX, A., DAVIS, F. P., & EWING, F. M. (1946). *P. gallinaceum* infection characterized by predominance of exo-erythrocytic forms. *Publ. Hlth. Rep., Wash.*, 62, (i), 921–8.

HAAS, V. H., WILCOX, A., & EWING, F. M. (1945). Infection of chick embryos with non-pigmented forms of *P. gallinaceum*. *J. nat. Malar. Soc.*, 4, 279–84.

—— (1947). Non-pigmented forms of *P. gallinaceum* in chick embryos: water color plates. *J. nat. Malar. Soc.*, 6, 122–3.

HAAS, V. H., WILCOX, A., LAIRD, R. L., EWING, F. M., & COLEMAN, N. (1948). Symposium on exoerythrocytic forms of malarial parasites. VI. Response of exoerythrocytic forms to alterations in the life-cycle of *P. gallinaceum*. *J. Parasit.*, 34, 306–20.

HACKETT, L. W. (1937). *Malaria in Europe: an ecological study.* (Heath Clark Lect. 1934.) London: Oxford Univ. Press.

HADDOW, A. J. (1952). Field and laboratory studies on an African monkey *Cercopithecus ascanius schmidti* Matschie. *Proc. zool. Soc. Lond.*, 122, 297–394.

HALLER, E. VON (1947). Ueber die Ursache und das Auftreten von Rückfällen bei der Malaria tertiana. *Med. Klinik.*, 42, 417–21.

HARRISON, W. S. (1909). On the cause of relapse in malaria. *J. R. Army med. Corps.*, 13, 647–9.

HAWKING, F. (1944). Tissue culture of malaria parasites (*P. gallinaceum*). *Lancet*, i, 693–4.

—— (1945). Growth of protozoa in tissue culture. I. *P. gallinaceum*, exoerythrocytic forms. *Trans. R. Soc. trop. Med. Hyg.*, 39, 245–63.

—— (1946). Growth of protozoa in tissue culture. II. *P. relictum*, exoerythrocytic forms. *Trans. R. Soc. trop. Med. Hyg.*, 40, 183–8.

—— (1948). Pre-erythrocytic stage in mammalian malaria parasites. *Nature, Lond.*, 161, 175.

—— (1951). Tissue culture of Plasmodia. *Brit. med. Bull.*, 8, 16–21.

—— (1953). Milk diet, *p*-aminobenzoic acid, and malaria (*P. berghei*). *Brit. med. J.*, i, 1201–2.

HAWKING, F., & HUNT, R. (1947). The *kochi* type of malaria parasite in monkeys. *Liber Jubilaris J. Rodhain* (suppl. to: *Ann. Soc. belge Méd. trop.*, 27), 251–6.

HAWKING, F., PERRY, W. L. M., & THURSTON, J. P. (1948a). Tissue forms of a malaria parasite, *P. cynomolgi*. *Lancet*, i, 783.

—— (1948b). Tissue forms of *P. cynomolgi*. Laboratory demonstration. *Trans. R. Soc. trop. Med. Hyg.*, 42, 10.

HAWKING, F., & THURSTON, J. P. (1952). Chemotherapeutic and other studies on pre-erythrocytic forms of simian malaria (*P. cynomolgi*). *Trans. R. Soc. trop. Med. Hyg.*, 46, 293–300.

HEGNER, R. W., & ESKRIDGE, L. C. (1938). Susceptibility of young red cells to the merozoites of avian plasmodia. *Amer. J. Hyg.*, 27, 471–92.

HEGNER, R. W., & WOLFSON, F. (1938a). *Toxoplasma*-like parasites in canaries infected with *Plasmodium*. *Amer. J. Hyg.*, 27, 212–20.

—— (1938b). Association of *Plasmodium* and *Toxoplasma*-like parasites in birds. *Amer. J. Hyg.*, 28, 437–54.

—— (1938c). The possibility of mixed infections in avian malaria. *Acta Conv. 3. trop. Morbis*, 2, 556–66.

—— (1939). Tissue-culture studies of parasites in reticulo-endothelial cells in birds infected with *Plasmodium*. *Amer. J. Hyg.*, 29 (sect. C), 83–7.

HENRY, CH. (1939). Pouvoir infestant du sang au cours de l'incubation du paludisme de la poule (*P. gallinaceum*) inoculé par moustiques. *Bull. Soc. Pat. exot.*, 32, 30–4.

HENSON, G. E. (1912). Aetiological factors in malarial recurrences. *Sth. med. J., Nashville*, 5, 450–7.

HERBIG-SANDREUTER, A. (1953). Untersuchungen über den Einfluss des Hohenklimas auf Huhnermalaria (*P. gallinaceum* Brumpt). *Acta trop., Basel*, 10, 1–27.

HERNANDEZ, T., MYATT, A. V., COATNEY, G. R., & JEFFERY, G. M. (1953). Studies in human malaria. XXXIV. Acquired resistance to pyrimethamine (Daraprim) by the Chesson strain of *P. vivax*. *Amer. J. trop. Med. Hyg.*, 2, 797–804.

HEWITT, R. I. (1940). Exo-erythrocytic bodies in canaries infected with a Mexican strain of *P. cathemerium*. *Amer. J. Hyg.*, 31 (sect. C), 61–6.

—— (1941). The distribution of avian malaria parasites (*P. relictum* and *P. cathemerium*) in visceral organs as compared with peripheral blood. *Amer. J. Hyg.*, 33 (sect. C), 54–68.

HITCHINGS, G. H. (1952). Daraprim as an antagonist of folic and folinic acids. *Trans. R. Soc. trop. Med. Hyg.*, 46, 467–73.

HOARE, C. A. (1924). *Hepatozoön adiei* n. sp., a blood parasite of an Indian eagle. *Trans. R. Soc. trop. Med. Hyg.*, 18, 63–6.

HÖRING, R. O. (1947). Induced and war malaria. *J. trop. Med. Hyg.*, 50, 150–9.

HUFF, C. G. (1930). *Plasmodium elongatum* n. sp., an avian malarial organism with an elongate gametocyte. *Amer. J. Hyg.*, **11**, 385–91.

—— (1938). Studies on the evolution of some disease-producing organisms. *Quart. Rev. Biol.*, **13**, 196–206.

—— (1942). Schizogony and gametocyte development in *Leucocytozoön simondi* and comparisons with *Plasmodium* and *Haemoproteus*. *J. infect. Dis.*, **71**, 18–32.

—— (1945). A consideration of the problem of evolution of malarial parasites. *Rev. Inst. Salubr. Enferm. trop., Méx.*, **6**, 253–8.

—— (1947). Life cycle of malaria parasites. *Annu. Rev. Microbiol.*, **1**, 43–60.

—— (1948a). Symposium on exoerythrocytic forms of malarial parasites. I. Introduction. *J. Parasit.*, **34**, 261–3.

—— (1948b). Exo-erythrocytic stages of malarial parasites. *Amer. J. trop. Med.*, **28**, 527–31.

—— (1948c). Periodos exo-eritrocíticos de los parásitos de la malaria. *Medicina, Méx.*, **28**, 25–33.

—— (1948d). Natural immunity and susceptibility of doves and pigeons to exo-erythrocytic and erythrocytic stages of *P. relictum*. *Proc. 4th int. Congr. trop. Med. Malar., Wash.*, **1**, 601–6.

—— (1949). Life cycles of malaria parasites with special reference to the newer knowledge of pre-erythrocytic stages. *Malariology* . . . Ed. by M. F. Boyd. Philadelphia & London : W. B. Saunders & Co., **1**, 54–64.

—— (1950). Letter to the Editor. *Trop. Med. News*, **7**, No. 3, 22–3.

—— (1951). Observations on the pre-erythrocytic stages of *P. relictum*, *P. cathemerium* and *P. gallinaceum* in various birds. *J. infect. Dis.*, **88**, 17–26.

—— (1952). Studies on the exo erythrocytic stages of *P. gallinaceum* during the " transition phase ". *Res. Rep. nav. med. Res. Inst.*, **10**, 397–414.

—— (1953a). Observations of *P. huffi* Muñiz, Soares and Batista. *Amer. J. trop. Med. Hyg.*, **2**, 620–3.

—— (1953b). Personal communication cited by M. K. Afridi. *5. Congr. int. Méd. trop. Palud.*, Istanbul, Rapports, **1**, 12.

—— (1953c). Personal communication.

—— (1954). Similarities between schizogony and sporogony in *Plasmodium*. *Res. Rep. nav. med. Res. Inst.*, **12**, 129.

HUFF, C. G., & BLOOM, W. (1935). A malarial parasite infecting all blood and blood forming cells of birds. *J. infect. Dis.*, **57**, 315–36.

HUFF, C. G., & COULSTON, F. (1944). The development of *P. gallinaceum* from sporozoite to erythrocytic trophozoite. *J. infect. Dis.*, **75**, 231–49.

—— (1946). The relation of natural and acquired immunity of various avian hosts to the cryptozoites and metacryptozoites of *P. gallinaceum* and *P. relictum*. *J. infect. Dis.*, **78**, 99–117.

—— (1948). Symposium on exo-erythrocytic forms of malaria parasites. II. A search for pre-erythrocytic stages of *P. vivax* and *P. cynomolgi*. *J. Parasit.*, **34**, 264–89.

HUFF, C. G., COULSTON, F., & CANTRELL, W. (1943). Malarial cryptozoites. *Science*, **97**, 286.

HUFF, C. G., COULSTON, F., LAIRD, R. L., & PORTER, R. J. (1947). Pre-erythrocytic development of *P. lophurae* in various hosts. *J. infect. Dis.*, **81**, 7–13.

HUFF, C. G., MARCHBANK, D. F., SAROFF, A. H., SCRIMSHAW, P. W., & SHIROISHI, T. (1950). Experimental infections with *P. fallax* Schwetz isolated from the Uganda tufted guinea fowl *Numidia meleagris major* Hartlaub. *J. nat. Malar. Soc.*, **9**, 307–19.

HUGHES, C. O., & BRACKETT, S. (1946). The prevention of sporozoite induced infections of *P. cathemerium* in the canary by metachloridine. *J. Parasit.*, **32**, 340–4.

INOKI, S. (1951a). Studies on the exo-erythrocytic schizogony of the malaria parasite. *Med. J. Osaka Univ.*, 2, 45–53.

—— (1951b). *Studies on the exo-erythrocytic schizogony of the malarial parasite. Med. J. Osaka Univ.*, 2, 407–19.

INOKI, S., OKUNO, Y., & AOYAMA, A. (1951). On the length of the asexual life cycle of *P. inui* var. *cyclopsis*. *Med. J. Osaka Univ.*, 2, 37–43.

IYER, P. V. S., SHORTT, H. E., & MENON, K. P. (1941). The stage of *P. gallinaceum* found in *the incubation period. Second observation. J. Malar. Inst. India*, 4, 179–80.

JACOBI, L. (1939). Beiträge zur Pathologie der Infektion des Huhnes mit *P. gallinaceum* (Brumpt). *Arch. exp. Path. Pharmak.*, 191, 482–91.

JACOBS, B., & SHORTT, H. E. (1951). *P. relictum* in the English blackbird. Blood and brain smears and sections showing exo-erythrocytic schizogony. *Trans. R. Soc. trop. Med. Hyg.*, 44, 356.

JACOBSEN, W. (1952). Discussion on the "Symposium on Daraprim". *Trans. R. Soc. trop. Med. Hyg.*, 46, 501–2.

JAMES, S. P. (1931a). Some general results of a study of induced malaria in England. *Trans. R. Soc. trop. Med. Hyg.*, 24, 477–525.

—— (1931b). *The use of plasmoquine in the prevention of malarial infections. Proc. R. Acad. Sci., Amst.*, Sect. Sci., 34, 1424–5.

—— (1937). Advances in knowledge of malaria since the war. *Trans. R. Soc. trop. Med. Hyg.*, 31, 263–80.

—— (1939). The incidence of exo-erythrocytic schizogony in *Plasmodium gallinaceum* in *relation to the mode of infection. Trans. R. Soc. trop. Med. Hyg.*, 32, 763–9.

JAMES, S. P., NICOL, W. D., & SHUTE, P. G. (1931). On the prevention of malaria with plasmoquine. *Lancet*, ii, 341–2.

—— (1936). Clinical and parasitological observations on induced malaria. *Proc. R. Soc. Med.*, 29, II, 879–94.

JAMES, S. P., & TATE, P. (1937a). New knowledge of the life cycle of malaria parasites. *Nature, Lond.*, 139, 545.

—— (1937b). Preparations illustrating the recently discovered cycle of avian malaria parasites in reticulo-endothelial cells. *Trans. R. Soc. trop. Med. Hyg.*, 31, 4–5.

—— (1938). Exo-erythrocytic schizogony in *P. gallinaceum* Brumpt, 1935. *Parasitology*, 30, 128–39.

JAMES, W. M. (1912). On relapses in malaria. *Proc. Canal Zone med. Ass.*, 5, pt. 1, 119–65.

JEFFERY, G. M. (1944). Investigations on the mosquito *transmission of P. lophurae* Coggeshall, 1938. *Amer. J. Hyg.*, 40, 251–63.

JEFFERY, G. M., WOLCOTT, G. B., YOUNG, M. D., & WILLIAMS, D. C., Jr. (1951). Exo-erythrocytic stages of *P. falciparum*. *J. Parasit.*, 37, No. 5, sect. 2 (suppl.), 25.

—— (1952). Exo-erythrocytic stages of *P. falciparum*. *Amer. J. trop. Med. Hyg.*, 1, 917–26.

JERACE, F. (1939). *Raro reperto ematico e parassitario nella malaria umana. Riv. Malariol.*, sez. 1, 18, 153–8.

KAMACHI, Y., & OH, K. (1943a). *Nettai Igaku*, 1, 248, quoted by INOKI, S. (1951a).

—— (1943b). *Nettai Igaku*, 1, 556, quoted by INOKI, S. (1951a).

KIKUTH, W. (1935). L'immunologie expérimentale et la chimiothérapie du paludisme. *Riv. Malariol.*, 14, sez. 1, suppl. 2, 71–80.

—— (1937a). Endotheliale Schizogonie bei Hühnermalaria (*P. gallinaceum* Brumpt 1935). *Zbl. Bakt.*, 1. Abt., Orig., 140, 227–36.

—— (1937b). Studien über die *Sporozoiten der Malariaparasiten. Festschr. B. Nocht.*, Hamburg, 240–7.

KIKUTH, W. (1938). Weiterentwicklung der Chemotherapie der Malaria. *Riv. Malariol.*, 17, sez. 1, 411–22.

—— (1939). Die Chemotherapie der Malaria auf Grund parasitologischer Erkenntnisse. *Münch. med. Wschr.*, 86, 362–5.

—— (1943). Zur Frage der Fruhjahrsrezidive der Malaria tertiana. *Z. ImmunForsch.*, 104, 148–157.

KIKUTH, W., & GIOVANELLA, A. (1933). Zur Frage der medikamentösen Malaria-prophylaxe auf Grund von experimentellen Untersuchangen an der Vogelmalaria. *Riv. Malariol.*, 12, sez. 1, 657–74.

KIKUTH, W., & MUDROW, L. (1937). Ueber pigmentlose Schizogonieformen der Vogelmalaria. *Klin. Wschr.*, 16, 1690–91.

—— (1938a). Malariaubertragungsversuche mit Blut und Organen sporozoiteninfizierter Kanarienvögeln. *Riv. Malariol.*, 17, 1–14.

—— (1938b). Die endothelialen Stadien der Malariaparasiten in Experiment und Theorie. *Zbl. Bakt.*, 1. Abt., Orig., 142, 113–32.

—— (1939a). Die Entwicklung der Sporozoiten von *P. cathemerium* im Kanarienvogel. *Zbl. Bakt.*, 1. Abt., Orig., 145, 81–8.

—— (1939b). Fruhstadien der Vogelmalariaparasiten nach Sporozoiteninfektion. *Klin. Wschr.*, 18, 1443–4.

—— (1939c). Chemotherapeutische Untersuchungen an den endothelialen Formen (E-stadien) des *P. cathemerium*. *Z. ImmunForsch.*, 95, 285–307.

—— (1940). Die Umwandlung der Sporozoiten in die endotheliale Phase der Malariaparasiten. *Riv. Malariol.*, 19, 1–15.

—— (1941a). Die endotheliale Phase der Malariaparasiten und ihre theoretische und praktische Bedeutung. *Ergebn. Hyg. Bakt.*, 24, 1–86.

—— (1941b). Ueber die Entwicklung der Sporozoiten der Malariaparasiten. *Zbl. Bakt.*, 1. Abt., Orig., 147, 284–8.

KIKUTH, W., & MUDROW-REICHENOW, L. (1947). Ueber kausalprophylaktisch bei Vogelmalaria wirksame Substanzen. *Z Hyg. Infektkr.*, 127, 151–65.

KNOCHE, E. (1941). Zum Problem der exo-erythrocytären Entwicklungsformen von *P. gallinaceum*. *Arch. exp. Path. Pharmak.*, 197, 240–51.

KNOPPERS, A. T. (1949). Two-fold quinine resistance of *P. gallinaceum* induced by regular administration of the drug. *Docum. neerl. indones. Morb. trop.*, 1, 55–66.

KRITSCHEWSKI, I. L., & MEERSON, J. S. (1932). Ueber die Bedeutung des retikulo-endothelialen Apparates bei Infektionskrankheiten. IX. Die Rolle des Retikuloendothelialsystems bei Vogelmalaria *Z. ImmunForsch.*, 76, 499–506.

LAIRD, M. (1951). *P. lygosomae* n. sp, a parasite of the New Zealand skink *Lygosoma moco*, (Gray, 1839). *J. Parasit.*, 37, 183–9.

—— (1953). *P. vaughani* Novy and MacNeal, 1904, in the New Hebrides; with a note on the occurrence of *elongatum*-type exo-erythrocytic schizogony in this species. *J. Parasit.*, 39, 357–64.

LAIRD, R. L., DUBIN, I. N., & DRINNON, V. P. (1950). The infection of chicks with pre-erythrocytic stages of *P. gallinaceum* grown in tissue culture. *J. nat. Malar. Soc.*, 9, 128–31.

LAMPEN, J. O., & JONES, M. J. (1946). The antagonism of sulfonamides by pteroylglutamic acid and related compounds. *J. biol. Chem.*, 164, 485–6.

LANZA, G. (1944). Documentazione di forme isotrope del parassita malarico nell' uomo. *Pathologica*, 36, 24–7.

LASTRA, I., & COATNEY, G. R. (1950). Transmission of *Haemoproteus columbae* by blood inoculation and tissue transplants. *J. nat. Malar. Soc.*, 9, 151–2.

LAUNOY, L. L. (1940). Sur quelques caractères de la maladie expérimentale à *P. gallinaceum* chez le jeune poulet. *Bull. Soc. Pat. exot.*, 33, 413–15.

LAVERAN, A. (1900). Au sujet de l'hématozoaire endoglobulaire du *Padda orizivora*. *C. R. Soc. Biol., Paris*, 52, 19–20.

LEVADITI, C. (1951). La phase exo-érythrocytaire des hématozoaires du paludisme. *Rev. Palud. Méd. trop.*, 9, 121–38.

LEVADITI, C., & SCHOEN, R. (1932). Sur un parasite sporulé du singe. *C. R. Soc. Biol., Paris*, 109, 343–6.

LEWERT, R. M. (1948a). Exo-erythrocytic infection by *P. gallinaceum* in blood-infected quinine-treated chicks, with special reference to the central nervous system. *Amer. J. Hyg.*, 48, 158–70.

—— (1948b). Alterations in the development of *P. gallinaceum* following passage through tissue culture. *Science*, 107, 250.

—— (1950a). Alterations in the cycle of *P. gallinaceum* following passage through tissue culture. I. Tissue culture studies. *Amer. J. Hyg.*, 51, 155–77.

—— (1950b). Alterations in the cycle of *P. gallinaceum* following passage through tissue culture. II. The behaviour of the strains during multiple passage through chicks. *Amer. J. Hyg.*, 51, 178–93.

LEWERT, R. M. (1952a). Nucleic acids in plasmodia and the phosphorus partition of cells infected with *P. gallinaceum*. *J. infect. Dis.*, 91, 125–44.

—— (1952b). Changes in nucleic acids and protein in nucleated erythrocytes infected with *P. gallinaceum* as shown by ultraviolet absorption measurements. *J. infect. Dis.*, 91, 180–3.

LIOIA, N. (1942). Ricerche sull' esistenza di un ciclo endoistiocitario dei plasmodi umani nei malarici cronici. *Riv. Parassit.*, 6, 173–4.

LLOYD, O. C., & SOMMERVILLE, T. (1949). The fate of sporozoites of *P. cynomolgi* injected into the skin of *Rhesus* monkeys. *J. Path. Bact.*, 61, 144–6.

LOEB, R. F., CLARK, W. M., COATNEY, G. R., COGGESHALL, L. T., DIEUAIDE, F. R., DOCHEZ, A. R., HAKANSSON, E. G., MARSHALL, E. K., Jr., MARVEL, C. S., MCCOY, O. R., SAPERO, J. J., SEBRELL, W. H., SHANNON, J. A., & CARDEN, G. A., Jr. (1947). Activity of a new antimalarial agent, pentaquine (SN 13276). *J. Amer. med. Ass.*, 132, 321–3.

MACCALLUM, W. G. (1898). Notes on the pathological changes in the organs of birds infected with haemocytozoa. *J. exp. Med.*, 3, 103–16.

MCGHEE, R. B. (1949a). The course of infection of *P. gallinaceum* in duck embryos. *J. infect. Dis.*, 84, 98–104.

—— (1949b). Pre-erythrocytic development of *P. gallinaceum* in avian embryos. *J. infect. Dis.*, 84, 105–10.

—— (1949c). The course of infection of *P. lophurae* in chick embryos. *J. Parasit.*, 35, 411–16.

MCLAY, K. (1922). Haematological investigations on malaria in Macedonia. *J. R. Army med. Corps.*, 38, 93–105.

MAIER, J. (1946). Screening tests for antimalarial activity. Code symbol C. In: Wiselogle, F. Y. (Ed.) *A survey of antimalarial drugs 1941–1945*, Ann Arbor, Mich.: J. W. Edwards, 1, 469–71.

MALARIA COMMISSION, 3rd General Report of (1933). *Quart. Bull. Hlth. Org., L. of N.*, 2, 181–285.

MANNABERG, J. (1894). The malarial parasites. In: *Two monographs on malaria. The New Sydenham Soc.*, 150, 235–428.

MANRESA, M. (1953). The occurrence of phanerozoites of *P. lophurae* in blood-inoculated turkeys. *J. Parasit.*, 39, 452–5.

MANWELL, R. D. (1929). Relapse in bird malaria. *Amer. J. Hyg.*, 9, 308–45.

MANWELL, R. D. (1935). How many species of avian malaria parasites are there? *Amer. J. trop. Med.*, **15**, 265–83.
—— (1939). *Toxoplasma* or exo-erythrocytic schizogony in malaria? *Riv. Malariol.*, **18**, 76–88.
—— (1940). Life cycle of *P. relictum* var. *matutinum*. *Amer. J. trop. Med.*, **20**, 859–66.
—— (1946). Bat malaria. *Amer. J. Hyg.*, **43**, 1–12.
—— (1947). Failure of *Aedes aegypti* and *Culex pipiens* to transmit *P. vaughani*. *J. Parasit.*, **33**, 167–9.
—— (1951a). Exoerythrocytic schizogony in *P. hexamerium*. *Amer. J. Hyg.*, **53**, 244.
—— (1951b). Leucocytozoa and other blood parasites of the purple grackle, *Quiscalus quiscala quiscala*. *J. Parasit.*, **37**, 301–6.
MANWELL, R. D., & GOLDSTEIN, F. (1939). Exo-erythrocytic stages in the asexual cycle of *P. circumflexum*. *Amer. J. trop. Med.*, **19**, 279–95.
MANWELL, R. D., & VOTER, M. A. (1939). Periodicity in the asexual cycle of *P. nucleophilum* with additional notes on this species. *Amer. J. trop. Med.*, **19**, 531–44.
MARCHIAFAVA, E., & BIGNAMI, A. (1894). On summer-autumn malarial fevers. In: *Two monographs on malaria*. *The New Sydenham Soc.*, **150**, 1–234.
MARSHALL, E. K., Jr., LITCHFIELD, J. T., Jr , & WHITE H. J. (1942). Sulfonamide therapy of malaria in ducks. *J. Pharmacol.*, **75**, 89–104.
MARTÍNEZ MUJICA, C. (1948). Reticulo-endoteliosis paludica. *Medicina, Méx.*, **28**, 417 ; 454 ; 459 ; 481.
MATHIS, C., & LEGER, M. (1909). Recherches sur le leucocytozoon de la poule. Périodicité des formes sexuées dans le sang. *C. R. Soc. Biol., Paris*, **67**, 688–90.
MATILLA, V., & APARICIO, J. (1943). Las formas " libres " de los parásitos en el paludismo humano. *Med. colon.*, **1**, 1–28.
MAYNE, B. (1933). The injection of mosquito sporozoites in malaria therapy. *Publ. Hlth Rep., Wash.*, **48**, 909–16.
—— (1937). Protracted incubation in malarial fever. Report of a case and a review of the literature. *Publ. Hlth Rep., Wash.*, **52**, 1599–1607.
MELLO, I. F. DE (1915). Preliminary note on a new haemogregarine found in the pigeon's blood. *Indian J. med. Res.*, **3**, 93–4.
MER, G. G., & GOLDBLUM, N. (1947). A haemosporidian of bats. *Nature, Lond.*, **159**, 444.
MEYER, H. (1947). Cultivation of the erythrocytic form of *P. gallinaceum* in tissue cultures of embryonic chicken brain. *Nature, Lond.*, **160**, 155–6.
—— (1949a). Cultivo de *P. gallinaceum* em culturas de tecido a partir de sangue infectado. II. *Rev. bras. Biol.*, **9**, 211–16.
—— (1949b). *The cultivation of protozoa in tissue cultures.* Monograph of the Instituto de Biofísica, Rio de Janeiro.
—— (1953). Private communication.
MEYER, H., & OLIVEIRA, M. X. DE (1947). Estudo morfológico da forma exo-erytrocitária do *P. gallinaceum* em culturas de tecido, I. *Rev. bras. Biol.*, **7**, 327–33.
MISSIROLI, A. (1933). Recerche sullo sviluppo dei parassiti malarigeni. *Riv. Malariol.*, **12**, 985–6.
—— (1934). Sullo sviluppo dei parassiti malarici. *Riv. Malariol.*, **13**, 539–52.
—— (1937a). Sullo sviluppo dei parassiti malarici. 3a nota. *Riv. Malariol.*, **16**, 99–107.
—— (1937b). Azione della chinina sui parassiti malarici durante l'incubazione. *Festschr. B. Nocht.*, *Hamburg*, 323–31.
—— (1938). Sullo sviluppo dei parassiti malarici. 4a nota. *Riv. Parassit.*, **2**, 39–43.
—— (1939). Sullo sviluppo dei parassiti malarici. *Riv. Parassit.*, **3**, 339–41.
—— (1940). Sullo sviluppo dei parassiti malarigeni. *Riv. Parassit.*, **4**, 69.

MISSIROLI, A. (1941a). Ueber die Entwicklung der Malariaparasiten. *Zbl. Bakt.*, 1. Abt., Orig., 146, 353–9.

—— (1941b). L'azione dei medicamenti sullo sviluppo degli sporozoiti. *Riv. Parassit.*, 5, 119–20.

—— (1943). Sullo sviluppo degli sporozoiti dei parassiti malarici. *Riv. Parassit.*, 7, 37–42.

MONIER, H. M. (1931). Essais thérapeutiques du 710 Fourneau dans quelques cas de paludisme. *Bull. Soc. Pat. exot.*, 24, 97–101.

MONTANARI, A. (1952). Sulla comparsa nel sangue periferico di elementi di origine diretta connettivale, di linfoblasti e di elementi atipici nel paludismo estivo-autunnale di prima invasione. *Riv. Malariol.*, 31, 153–6.

MORRIONE, T. G., & MAMELOK, H. L. (1952). Observations on the persistence of hepatic glycogen after death. *Amer. J. Path.*, 28, 497–502.

MOSNA, E. (1940). Sul conportamento delle forme del ciclo eso-eritrocitico del *P. gallinaceum*. *Riv. Parassit.*, 4, 241–8.

—— (1947). Raro reperto di forme endoistiocitarie di *P. gallinaceum* nel sangue periferico. *Riv. Parassit.*, 8, 19–22.

MOST, H., KANE, C. A., LAVIETES, P. H., LONDON, I. M., SCHROEDER, E. F., & HAYMAN, J. M. (1946). Combined quinine-plasmochin treatment of *vivax* malaria: effect on relapse rate. *Amer. J. med. Sci.*, 212, 550–60.

MUDROW, L. (1940). Klinische und parasitologische Befunde und chemotherapeutische Ergebnisse bei der Hühnermalaria. *Arch. Schiffs- u. Tropenhyg.*, 44, 257–75.

—— (1942a). Ueber die Entwicklung der Sporozoiten bei verschiedenen Vogelmalaria-arten. *Riv. Malariol.*, 21, 29–46.

—— (1942b). *Zur Sporozoitenentwicklung verschiedener Vogelmalaria-arten. Riv. malariol.*, 21, 382–5.

MUDROW, L., & REICHENOW, E. (1944). Endotheliale und erythrocytäre Entwicklung von *Plasmodium praecox*. *Arch. Protistenk.*, 97, 101–70.

MUDROW-REICHENOW, L. (1949). Unser heutiges Wissen von der Plasmodienentwicklung im Wirbeltier. *Z. Tropenmed. u. Parasit.*, 1, 113–52.

—— (1950). Ergebnisse und Probleme der Malariaforschung. *Riv. Malariol.*, 29, 133–48.

—— (1951). Ueber die chemotherapeutische Beeinflussbarkeit des *P. berghei* Vincke u. Lips. *Z. Tropenmed. Parasit.*, 2, 471–85.

MUDROW-REICHENOW, L., & KIKUTH, W. (1949). Neue Forschungsergebnisse über die Biologie der Malariaparasiten. *Dtsch. med. Wschr.*, 74, 759–63.

MUDROW-REICHENOW, L., & REICHENOW, E. (1949). Die Entwicklung von *P. cathemerium* im Endothel und im Blut des Kanarienvogels. *Zool. Jb.*, 70, 129–68.

MULLIGAN, H. W. (1947). Mammalian malaria enquiry. *Rep. sci. Adv. Bd. Indian Res. Fund Ass.*, 1946, pp. 9–14.

MULLIGAN, H. W., SOMMERVILLE, T., & LLOYD, O. C. (1949). Observations on the infectivity of tissues of *Macaca mulatta* during the incubation period following exposure to infection with sporozoites of *P. cynomolgi*. *Indian J. Malar.*, 3, 211–34.

MUNIZ, J., SOARES, R., & BATISTA, S. (1950). Sôbre um *Plasmodium* com estadios exoeritrocitários do tipo *gallinaceum*, parasito do *Ramphastos toco* Müller, 1776. *5. Congr. int. Microbiol., Rio de Janeiro, Resumas dos Trabalhos*, p. 153.

—— (1951). On a new species of *Plasmodium* parasite of *Ramphastos toco* Müller 1776—*P. Huffi* n. sp. *Rev. bras. Malariol.*, 3, 339–62.

NAUCK, E. G. (1953). Immunitätsprobleme bei Malaria. *Z. Tropenmed. Parasit.*, 4, 285–98.

NEEB, H. M. (1910). The parthenogenesis of the female crescent body. *J. trop. Med. Hyg.*, 13, 98–102.

NELSON, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *J. biol. Chem.*, **153**, 375–80.

NUCCIOTTI, L. (1942). Ricerche sull' esistenza di forme endoistiocitarie dei plasmodi umani nel midollo sternale di malarici primitivi. *Riv. Parassit.*, **6**, 229–35.

OBERLÉ, G. (1945). Recherches sur les formes extraérythrocytaires du paludisme humain à *P. vivax*. *Bull. Soc. Pat. exot.*, **38**, 27–37.

ODDO, F. G., & BRUNO-SMIRAGLIA, C. (1951). La persistenza dell' infezione da *P. gallinaceum*: pigmento melanico, parassiti endo- ed eso-eritrocitici. *Riv. Parassit.*, **12**, 215–26.

OLIVEIRA, M. X. DE (1950). Cultivato do *P. gallinaceum* em cultura de tecido. III. Estudo das formas responsáveis pela obtenção da cultura a partir de sangue infectado. *Rev. bras. Biol.*, **10**, 115–19.

PARAENSE, W. L. (1943). Aspectos parasitários observados no local inoculado com esporozoitos de *P. gallinaceum*. (Nota preliminar.) *Mem. Inst. Osw. Cruz*, **38**, 353–9.

—— (1945). Sobre a evolução dos plasmodios do reticulo-endotelio. *Med. Cirurg. Pharm.*, **108**, 107.

—— (1946). Ação patogênica das formas exo-eritrocitárias do *P. gallinaceum*. 1. Investigações preliminares, 2. Prova terapêutica da ação patogênica. *Mem. Inst. Osw. Cruz*, **44**, 147–92.

—— (1947a). Estudios sôbre o ciclo exoeritrocitário do *P. gallinaceum*. *Mem. Inst. Osw. Cruz*, **45**, 101–18.

—— (1947b). Ação patogênica das formas exoeritrocitárias do *P. gallinaceum*. 3. Algumas caracteristicas das hemacias nas infecções tratadas com quinina. *Mem. Inst. Osw. Cruz*, **45**, 345–59.

—— (1947c). Obervações preliminares sôbre o ciclo exoeritrocitário do *P. juxtanucleare* Versiani e Gomes, 1941. *Mem. Inst. Osw. Cruz*, **45**, 813–24.

—— (1952a). Observations on a Brazilian strain of *P. circumflexum*. *Mem. Inst. Osw. Cruz*, **50**, 211–41.

—— (1952b). Pre erythrocytic parasites and immunity. *Trans. R. Soc. trop. Med. Hyg.*, **46**, 678

PARAENSE, W. L., & SILVA, E. M. DA (1941). Esquizogonia exoeritrocitária do *P. falciparum*. *Hospital, Rio de J.*, **20**, 99.

PARAENSE, W. L., MEYER, H., & MENEZES, V. (1942). Estudos sôbre *P. gallinaceum* comportamento dos esporozoitos em cultura de baco embrionario. *Rev. bras. Biol.*, **2**, 89–94.

PARISE, N., & LUCREZI, G. (1941). Ricerche sulla malaria cronica. *Riv. Malariol.*, sez. 1, **20**, 301–8.

PELAEZ, D., PEREZ REYES, R., & BARRERA, A. (1948). Estudios sobre hematozoarios. I. *Plasmodium mexicanum* Thompson & Huff, 1944, en sus huespedes naturales. *An. Esc. nac. Cienc. biol., Méx.*, **5**, 197–215.

PESSÔA, S. B., & CORRÊA, R. R. (1929). Nota sôbre toxoplasmas dos passaros. *Ann. paulist. Med. Cir.*, Anno. 17, **20**, 103–6.

PICCIOLI, A. (1941). Sul reperto di parassiti malarici nella biopsia midollare. *Sperimentale*, **95**, 789–92.

PORTER, R. J. (1942). The tissue distribution of exoerythrocytic schizonts in sporozoite-induced infections with *P. cathemerium*. *J. infect. Dis.*, **71**, 1–17.

—— (1948). Symposium on exoerythrocytic forms of malarial parasites. V. Studies in tissue culture of exoerythrocytic schizogony in avian malarial parasites. *J. Parasit.*, **34**, 300–5.

PORTER, R. J., & HUFF, C. G. (1940). Review of the literature on exo-erythrocytic schizogony in certain malarial parasites and its relation to the schizogonic cycle in *P. elongatum*. *Amer. J. trop. Med.*, **20**, 869–88.

PORTER, R. J., LAIRD, R. L., & DUSSEAU, E. M. (1952). Studies on malarial sporozoites. I. Effect of various environmental conditions. *Exp. Parasit.*, 1, 229–44.

PRATT, I. (1941). The effect of *Eimeria tenella* (Coccidia) upon the glycogen stores of the chicken. *Amer. J. Hyg.*, 34 (sect. C), 54–61.

PURCHASE, H. S. (1942). Turkey malaria. *Parasitology*, 34, 278–83.

QUATTRIN, N. (1941). Sul ciclo eso-eritrocitico del parassita malarico nel midollo sternale umano. *Riv. Parassit.*, 5, 17–23.

RAFFAELE, G. (1932). Sulle cosiddette toxoplasmosi dei passeri. *Riv. Malariol.*, 11, 825–38.

—— (1934a). Un ceppo italiano di *P. elongatum*. *Riv. Malariol.*, sez. 1, 13, 332–7.

—— (1934b). Sul comportamento degli sporozoiti nel sangue dell' ospite. *Riv. Malariol.*, sez. 1, 13, 395–403.

—— (1936a). Il doppio ciclo schizogonico di *P. elongatum*. *Riv. Malariol.*, sez. 1, 15, 309–17.

—— (1936b). Presumibili forme iniziali di evoluzione di *P. relictum*. *Riv. Malariol.*, sez. 1, 15, 318–24.

—— (1936c). Potere infettante del sangue durante l'incubazione della malaria aviaria. *Riv. Malariol.*, sez. 1, 15, 77–87.

—— (1937a). Ancora sul ciclo schizogonico di *P. elongatum*. *Riv. Malariol.*, sez. 1, 16, 79–83.

—— (1937b). Sullo sviluppo iniziale dei parassiti malarici nell' ospite vertebrato. *Riv. Malariol.*, sez. 1, 16, 185–98.

—— (1937c). Ricerche sul ciclo di evoluzione iniziale dei parassiti malarici umani. *Riv. Malariol.*, sez. 1, 16, 413–18.

—— (1938a). La fase primaria dell' evoluzione monogonica dei parassiti malarici. *Riv. Malariol.*, sez. 1, 17, 331–43.

—— (1938b). La fase primaria dell' evoluzione monogonica dei parassiti malarici. *Acta Conv. 3. trop. Morbis*, 2, 545–55.

—— (1938c). Recensione. *Riv. Malariol.*, sez. II, 17, 8–9.

—— (1938d). Evoluzione di *Plasmodium*, *Toxoplasma* ed altri microrganismi negli organi interni dei vertebrati. *Riv. Malariol.*, sez. I, 17, 85–106.

—— (1939). La fase apigmentata di evoluzione dei plasmodidi. *Arch. Zool. ital., Torino*, 26, 94–120.

—— (1940a). Recensioni—Avvertenza. *Riv. Malariol.*, sez. II, 19, 1–4.

—— (1940b). Ulteriori ricerche sulla fase monogonica primaria dei plasmodidi nell' uomo e negli uccelli. *Riv. Malariol.*, 19, 193–225.

—— (1941). Moderne vedute sulla biologia dei parassiti della malaria. Parte. I[a]. *Scientia, Bologna*, 70, 162–6.

—— (1942). Moderne vedute sulla biologia dei parassiti della malaria. Parte II[a]. *Scientia, Bologna*, 71, 11–18.

—— (1944a). L'infezione malarica e le nuove ricerche sulla biologia del parassita. I. *Riv. Malariol.*, 23, 73–86.

—— (1944b). L'infezione malarica e le nuove ricerche sulla biologia del parassita. *Riv. Malariol.*, 23, 105–18.

—— (1946a). Modern views on the biology of malaria parasites. *Acta trop., Basel.*, 3, 29–40.

—— (1946b). L'esperimento del Generale Fairley in Australia. *Riv. Malariol.*, 25, 36–53.

—— (1951). Le phase extra-érythrocytaire des parasites du paludisme chez les animaux et chez l'homme. *Sci. med. Ital.*, 2, 631–58.

—— (1952). Le cycle exo-érythrocytaire des plasmodies et ses rapports avec la thérapie du paludisme. *Riv. Malariol.*, 31, 135–43.

RAY, H. N. (1949). Exoerythrocytic schizogony in *Plasmodium* sp. in the Himalayan flying squirrel, *Petaurista inornatus* (Geoffrey). A preliminary note. *Proc. nat. Inst. Sci. India*, 15, 241–4.

Redmond, W. B., & Fincher, E. L. (1949). Exo-erythrocytic forms in relation to paludrine administration in pigeons infected with *P. relictum*. *J. Parasit.*, 35, No. 6, sect. 2 (suppl.), 25.

Refaat, M. A. (1953). Personal communication.

Reichenow, E. (1939). Die endothelialen Entwicklungsformen der Malariaparasiten im Lichte der Phylogenie der Haemosporidien. *Proc. 3rd int. Congr. Microbiol.*, p. 139.

—— (1947). Die Entwicklung der Malariaplasmodien im Vogelkörper. *Zbl. Bakt.*, 1. Abt., Orig., 152, 272–84.

Reichenow, E., & Mudrow, L. (1943). Der Entwicklungsgang von *P. praecox* im Vogelkorper. *Dtsch. tropenmed. Z.*, 47, 289–99.

Rice, J. B. (1932). Experiments on plasmoquine as a prophylactic among West African negroes exposed to bites of *A. costalis* infected with subtertian malaria. *Ann. trop. Med. Parasit.*, 26, 555–76.

Richeson, E. M., Highman, B., Greenberg, J., & Coatney, G. R. (1953). Strain difference in *P. gallinaceum* Brumpt. V. Further characteristics of the BI Strain. *Amer. J. Hyg.*, 57, 114–24.

Ritis, F. de (1940). Sul docorso dell' infezione da *P. gallinaceum* Brumpt, 1935. *Riv. Parassit.*, 4, 61–8.

Rodhain, J. (1938a). Schizogonie sans pigment chez les pingouins infectés de *P. praecox* (*relictum*). *C. R. Soc. Biol., Paris*, 127, 368–72.

—— (1938b). Schizogonie sans pigment chez un pingouin expérimentalement infecté de *P. praecox* (*relictum*). *C. R. Soc. Biol., Paris*, 127, 838–40.

—— (1939). L'infection à *P. relictum* chez les pingouins. *Ann. Parasit. hum. comp.*, 17, 139–57.

—— (1953). Contribution à l'étude de l'*Hepatocystis* (*Plasmodium*) *epomophori* Rodhain. *Ann. Soc. belge Méd. trop.*, 33, 285–92.

Rodhain, J., Gavrilov, W., & Cowez, S. (1940). Essais d'infection des cultures de tissus d'embryon de poulet par les sporozoïtes de *P. gallinaceum*. *C. R. Soc. Biol., Paris*, 134, 261–4.

Rollo, I. M. (1952). Daraprim—experimental chemotherapy. *Trans. R. Soc. trop. Med. Hyg.*, 46, 474–84.

Ross, R., & Thomson, D. (1910). Some enumerative studies on malarial fever. *Ann. trop. Med. Parasit.*, 4, 267–306.

Ruge, H. (1936a). Neuzeitliche Probleme der Malariaforschung. *Dtsch. med. Wschr.*, 62 (ii), 1869–72.

—— (1936b). Zur Frage der James'schen Sporozoitentheorie. *Z. Hyg.*, *InfektKr.*, 118, 724–37.

Ruhe, D. S., Cooper, W. C., Coatney, G. R., & Josephson, E. S. (1949). Studies in human malaria. XV. The therapeutic action of Pamaquin (Plasmochin) against St. Elizabeth strain *vivax* malaria. *Amer. J. Hyg.*, 49, 367–73.

Russell, P. F., Mulligan, H. W., & Mohan, B. N. (1941). Specific agglutinogenic properties of inactivated sporozoites of *P. gallinaceum*. *J. Malar. Inst. India*, 4, 15–24.

Russell, P. F., & Nono, A. M. (1932). Avian malaria studies. VII. Plasmochin as a prophylactic drug in sporozoite infections of avian malaria. *Philip. J. Sci.*, 49, 595–625.

Sanctis Monaldi, T. de (1935). Ricerche sulla malaria sperimentale da inoculazione di sporozoiti. *Riv. Malariol.*, sez. I, 14, 344–51.

Sanctis Monaldi, T. de, & Raffaele, G. (1953) Sulla fase negativa del sangue e nell' infezione da *P. vivax*. *Riv. Malariol.*, 32, 1–10.

Sandosham, A. A. (1951). Recent advances in our knowledge of the parasitology of malaria. *Proc. Alumni Ass. King Edw. VII Coll. Med., Singapore*, 4, 167–73.

Schaudinn, F. (1903). Studien über krankheitserregende Protozoen. *Arb. Kais. Gsndhts-amte.*, 19, 169–250.

Scheng, T. D. (1943). Zur Wirkung von Arzneistoffen auf die exoerythrocytären Entwicklungsformen der Plasmodien. *Arch. exp. Path. Pharmak.*, 201, 502–19.

Schingareff, A. J. (1906). Des hémosporidies des chauves-souris. *Arch. Sci. biol., St. Pétersb.*, 12, 181.

Schmidt, L. H. (1953). Personal communication.

Schmidt, L. H., Fradkin, R., Squires, W., & Genther, C. S. (1948). Malaria chemotherapy : 2. The response of sporozoite-induced infections with *P. cynomolgi* to various antimalarial drugs. *Fed. Proc.*, 7, 253–4.

Schmidt, L. H., & Genther, C. S. (1953). The antimalarial properties of 2, 4-diamino-5-p-chlorophenyl-6-ethylpyrimidine (Daraprim). *J. Pharmacol.*, 107, 61–91.

Schmidt, L. H., Genther, C. S., Fradkin, R., & Squires, W. (1949). Development of resistance to chlorguanide (paludrine) during treatment of infections with *P. cynomolgi*. *J. Pharmacol.*, 95, 382–98.

Schneider, J., & Schneider, C. R. (1950). *P. berghei.* Absence "d'incubation" au cours du passage de souris à souris par voie intrapéritonéale. *Bull. Soc. Pat. exot.*, 43, 324–32.

Schüffner, W. A. P., Korteweg, P. C., & Swellengrebel, N. H. (1929). Experimental malaria with protracted incubation period. *Proc. R. Acad. Sci. Amst.*, 32, 903–11.

Schulemann, W. (1940). Zur Pathologie der Malaria. *Dtsch. med. Wschr.*, 66, 253–6.

—— (1942). Zur Weiterentwicklung der Malariasporozoiten im Warmblüter. *Dtsch. med. Wschr.*, 68, 374–5.

Schulemann, W., & Knoche, E. (1941). Zum Problem der exo-erythrocytären Entwicklungsformen von *P. gallinaceum*. *Arch. exp. Path. Pharmak.*, 197, 227–39.

Schulemann, W., & Spies, K. (1940). Zu Ursprung und Entwicklung der pigmentfreien Formen der Malariaparasiten. *Dtsch. med. Wschr.*, 66, 404–5.

Schwetz, J. (1933). Contribution à l'étude des parasites malariens (*Plasmodium*) des singes inférieurs africains. *Zbl. Bakt.*, 1. Abt., Orig., 130, 111–20.

—— (1938). Schizonts in endothelial cells in monkey malaria. *Trans. R. Soc. trop. Med. Hyg.*, 31, 470–1.

Sergent, Ed. (1940). Parasites des paludismes et toxoplasmes. *Arch. Inst. Pasteur Algér.*, 18, 374–401.

—— (1941). Les Plasmodium du paludisme parasitent-ils d'autres cellules que les globules rouges? *Pr. méd.*, 49, 649–51.

—— (1949a). Contribution à l'étude du second cycle évolutif insexué des *Plasmodium* chez les paludéens. *Arch. Inst. Pasteur Algér.*, 27, 211–49.

—— (1949b). Sur les deux cycles évolutifs insexués des *Plasmodium* chez les paludéens. *C. R. Acad. Sci.*, 229, 455–8.

Sergent, Ed., & Béguet, M. E. (1914). De l'immunité dans le paludisme des oiseaux. Les pigeons guéris de l'infection à *Haemoproteus columbae* ne sont pas immunisés contre elle. *C. R. Soc. Biol., Paris*, 77, 21–3.

Sergent, Ét., & Sergent, Ed. (1921). Avantages de la quininisation préventive démontrés et précisés expérimentalement. *Ann. Inst. Pasteur*, 35, 125–41.

—— (1922). Étude expérimentale du paludisme des oiseaux (*Plasmodium relictum*). Suite des recherches sur l'action de la quinine. (XXIII[e]. note.) *Arch. Insts. Pasteur Afr. N.*, 2, 320–9.

Sergent, Ét., Sergent, A., Trensz, F., & Vogt, D. (1932). Essais de traitement du paludisme à *P. praecox* par le 710 Fourneau, seul ou associé à la quinine, chez des paludéens d'Algérie. *Arch. Inst. Pasteur. Algér.*, 10, 1–21.

Shortt, H. E. (1948a). The pre-erythrocytic cycle of *P. cynomolgi*. *Proc. 4th Congr. trop. Med. Malar.*, **1**, 607–17.

—— (1948b). The life cycle of *P. cynomolgi* in its insect and mammalian hosts. *Trans. R. Soc. trop. Med. Hyg.*, **42**, 227–30.

—— (1950). Recent advances in our knowledge of the malaria parasite. *Brit. med. J.*, ii, 606–8.

—— (1951a). History of recent research on tissue phases of the malaria parasite at the London School of Hygiene & Tropical Medicine. *Trans. R. Soc. trop. Med. Hyg.*, **45**, 175–88.

—— (1951b). Presumed 3-day old pre-erythrocytic schizont of *P. cynomolgi*. *Trans. R. Soc. trop. Med Hyg.*, **44**, 355.

Shortt, H. E., & Bray, R. S. (1952). Large schizont of *P. cynomolgi* 15 days after infection by mosquito. *Trans. R. Soc. trop. Med. Hyg.*, **46**, 2.

Shortt, H. E., Bray, R. S., & Cooper, W. (1954). Further notes on the tissue stages of *P. cynomolgi*. *Trans. R. Soc. trop. Med. Hyg.*, **48**, 122–31.

Shortt, H. E., & Cooper, W. (1947). Staining of microscopical sections containing protozoal parasites by modification of McNamara's method. *Trans. R. Soc. trop. Med. Hyg.*, **41**, 427–8.

Shortt, H. E., Cooper, W., & Bray, R. S. (1953). Two-day old pre-erythrocytic forms of *P. cynomolgi*. *Trans. R. Soc. trop. Med. Hyg.*, **47**, 11.

Shortt, H. E., Fairley, N. H., Covell, G., Shute, P. G., & Garnham, P. C. C. (1949). The pre-erythrocytic stage of *P. falciparum*. A preliminary note. *Brit. med. J.*, ii, 1006–8.

—— (1951). The pre-erythrocytic stage of *P. falciparum*. *Trans. R. Soc. trop. Med. Hyg.*, **44**, 405–19.

Shortt, H. E., & Garnham, P. C. C. (1948a). Pre-erythrocytic stage in mammalian malaria parasites. *Nature, Lond*, **161**, 126.

—— (1948b). The exoerythrocytic parasites of *P. cynomolgi*. *Trans. R. Soc. trop. Med. Hyg.*, **41**, 705.

—— (1948c). The pre-erythrocytic development of *P. cynomolgi* and *P. vivax*. *Trans. R. Soc. trop. Med. Hyg.*, **41**, 785–95.

—— (1948d). Successive stages in the pre-erythrocytic cycle of *P. cynomolgi* from the 5th to the 8th days of the inoculation period. Laboratory demonstration. *Trans. R. Soc. trop. Med. Hyg.*, **42**, 7.

—— (1948e). Demonstration of a persisting exo-erythrocytic cycle in *P. cynomolgi* and its bearing on the production of relapses. *Brit. med. J.*, i, 1225–8.

—— (1949). Earliest form seen to date of the pre-erythrocytic cycle of *P. cynomolgi*—4th day after the bite of the mosquito. Laboratory demonstration. *Trans. R. Soc. trop. Med. Hyg.*, **42**, 321.

Shortt, H. E., Garnham, P. C. C., Covell, G., & Shute, P. G. (1948). The pre-erythrocytic stage of human malaria *P. vivax*. *Brit. med. J.*, i, 547.

Shortt, H. E., Garnham, P. C. C., & Malamos, B. (1948). The pre-erythrocytic stage of mammalian malaria. *Brit. med. J.*, i, 192–4.

Shortt, H. E., & Malamos, B. (1947). Demonstration. *Trans. R. Soc. trop. Med. Hyg.*, **40**, 357.

Shortt, H. E., Menon, K. P., & Iyer, P. V. S. (1940). The form of *P. gallinaceum* present in the incubation period of infection. *Indian J. med. Res.*, **28**, 273–6.

Shute, P. G. (1946). Latency and long-term relapse in benign tertian malaria. *Trans. R. Soc. trop. Med. Hyg.*, **40**, 189–200.

—— (1953). Personal communication.

Shute, P. G., & Maryon, M. (1948). The gametocytocidal action of Paludrine upon infections of *Plasmodium falciparum*. *Parasitology*, **38**, 264–70.

Shute, P. G., & Maryon, M. (1954). The effect of pyrimethamine (Daraprim) on the gametocytes and oocysts of *Plasmodium falciparum* and *Plasmodium vivax*. *Trans. R. Soc. trop. Med. Hyg.*, 48, 50–63.

Sicault, G., & Messerlin, A. (1937) Vues nouvelles sur la maladie palustre et les thérapeutiques stérilisantes du paludisme. *Riv. Malariol.*, sez. I, 16, 305–23.

—— (1938). La maladie palustre. Réticulo-endothéliose parasitaire. *Pr. méd.*, 46 (ii), 1419–23.

Simpson, M. L. (1944). Exo-erythrocytic stages of *P. durae*. *J. Parasit.* 30, 177–8.

Sinton, J. A. (1946). Discussion. *Trans. R. Soc. trop. Med. Hyg.*, 40, 154.

Sinton, J. A., & Bird, W. (1928). Plasmoquine in the treatment of malaria. *Indian J. med. Res.*, 16, 159–77.

Sinton, J. A., Smith, S., & Pottinger, D. (1930). Further researches into the treatment of chronic benign tertian malaria with plasmoquine and quinine. *Indian J. med. Res.*, 17, 793–814.

Somogyi, M. (1945). A new reagent for the determination of sugars. *J. biol. Chem.*, 160, 61–8.

—— (1952). Notes on sugar determination. *J. biol. Chem.*, 195, 19–23.

Soskin, S., & Levine, R. (1946). *Carbohydrate metabolism.* Chicago: University of Chicago Press.

Spanedda, A., & Floris, M. (1945). Sulle forme E. E. del parassita malarico nell' uomo. *Riv. Malariol.*, 24, 177–90.

Spinks, A. (1946). The pharmacology of paludrine in animals. *Trans. R. Soc. trop. Med. Hyg.*, 40, 3–4.

Stephen, J. M. L., Tonkin, I. M., & Walker, J. (1945). Antimalarial activity in tetrahydroacridones and related substances. *Nature, Lond.*, 156, 629.

—— (1947). Tetrahydroacridones and related compounds as antimalarials. *J. chem. Soc.*, ii, 1034–9.

Swellengrebel, N. H., & Buck, A. de (1931). Prophylactic use of plasmoquine in a dosage warranting reasonable safety for routine treatment. *Proc. R. Acad. Sci., Amst.*, 34, 1216–20.

—— (1938). *Malaria in the Netherlands.* Scheltema and Holkema Ltd., Amsterdam.

Taddia, L. (1938). Plasmodidi e corpi *Toxoplasma*-simili nei passeri del Veneto. *Riv. Malariol.*, sez. 1., 17, 237–41.

Taddia, L., & Viero, G. (1940). Ricerche sulle fasi esoeritocitiche del *Plasmodium relictum*. *Riv. Parassit.*, 4, 45–9.

Taliaferro, W. H. (1944). Immunity in malaria. *Amer. J. clin. Path.*, 14, 593–7.

Taliaferro, W. H., & Taliaferro, L. G. (1947). Asexual reproduction of *P. cynomolgi* *J. infect. Dis.*, 80, 78–104.

Tarejev, E. (1935). Efficacité des dérivés synthétiques antipaludéens fabriqués en U.R.S.S. Note dactylographiée. Cited in: *Quart. Bull. Hlth Org. L. o. N.*, 1937, 6, 917.

Tarsitano, A., & Lucrezi, G. (1939). Reperti di forme apigmentate del parassita malarico. *Arch. ital. Sci. med. colon.*, 20, 65–9.

Tate, P., & Vincent, M. (1933). The action of plasmoquine on mosquito-induced malaria of birds. *Parasitology*, 25, 96–101.

Telcharov, L., & Todorowa, M. (1950). Le foie des paludéens. *Sem. Hôp. Paris*, 26, 2072–5.

Terzian, L. A. (1941). Studies on *P. lophurae*, a malarial parasite in fowls. I. Biological characteristics. *Amer. J. Hyg.*, 33 (sect. C), 1–22.

Terzian, L. A., Stahler, M., & Weathersby, A. B. (1949). The action of antimalarial drugs in mosquitoes infected with *Plasmodium gallinaceum*. *J. infect. Dis.*, 84, 47–55.

THOMPSON, P. E. (1945). The effects of sulfonamide diets upon infections of *P. elongatum* in canaries. *J. infect. Dis.*, 76, 15–19.

—— (1946). Effects of quinine on saurian malarial parasites. *J. infect. Dis.*, 78, 160–6.

THOMPSON, P. E., & HUFF, C. G. (1944a). A saurian parasite *P. mexicanum* n. sp. with both *elongatum*- and *gallinaceum*-types of exo-erythrocytic stages. *J. infect. Dis.*, 74, 48–67.

THOMSON, J. D. (1917). Notes on malaria. *J. R. Army med. Corps*, 29, 379–411.

THURSTON, J. P. (1953). The chemotherapy of *P. berghei*. I. Resistance to drugs. *Parasitology*, 43, 246–52.

TOKURA, N., & KAWAHATA, H. (1951). Observations of *P. elongatum* from Japanese wild birds. *Yokohama. med. Bull.*, 2, 85–8.

TOMLINSON, W. J., & GROCOTT, R. G. (1944). A simple method of staining malaria protozoa and other parasites in paraffin sections. *Amer. J. clin. Path.*, 14, 316–26.

TONKIN, I. M. (1946). The testing of drugs against exoerythrocytic forms of *P. gallinaceum* in tissue culture. *Brit. J. Pharmacol.*, 1, 163–73.

TONKIN, I. M., & HAWKING, F. (1947). Growth of protozoa in tissue culture. IV. *P. lophurae*, exoerythrocytic forms *in vivo* and *in vitro*. *Trans. R. Soc. trop. Med. Hyg.*, 41, 407–14.

TREMBLEY, H. L., GREENBERG, J., & COATNEY, G. R. (1951a). Strain differences in *P. gallinaceum* Brumpt. II. Experiences with the sporozoite and single oocyst passage of the BI strain. *J. nat. Malar. Soc.*, 10, 68–75.

—— (1951b). Strain differences in *P. gallinaceum* Brumpt. III. The spontaneous conversion of a phanerozoite-producing strain S. P. to a phanerozoite-less M. strain through mosquito passage. *J. nat. Malar. Soc.*, 10, 76–81.

—— (1951c). Strain differences in *P. gallinaceum* Brumpt. IV. Experiences with the blood passage of the phanerozoite-less M. strain. *J. nat. Malar. Soc.*, 10, 82–9.

TULLIS, J. L. (1947). The distribution of exoerythrocytic parasites and the tissue reaction caused by blood-induced *P. gallinaceum* infection in chicks. *Amer. J. trop. Med.*, 27, 21–9.

UEGAKI, J. (1930). Untersuchungen uber die Blutprotozoen von Vögeln der Sudsee. *Arch. Protistenk.*, 72, 74–90.

UNGO-MUGDAN, A. (1938). La reazione nucleare di Feulgen negli stadi exo-eritrocitici del *P. gallinaceum* Brumpt, 1935. *Riv. Parassit.*, 2, 323–6.

—— (1939). Sul comportamento degli stadi exo-eritrocitici del *P. gallinaceum* nelle infezioni con sporozoiti e con sangue. *Riv. Parassit.*, 3, 329–33.

VERNEY, L. (1938a). Recensione. *Riv. Malariol.*, sez. II, 17, 1–3.

—— (1938b). Sullo sviluppo dei parassiti malarici. *Riv. Malariol.*, sez. II, 17, 365–7.

—— (1938c). Lo sviluppo dei parassiti malarici nel reticuloendotelio. *Ann. Igiene*, 48, 26–40.

—— (1938d). Le prime fase di sviluppo dei parassiti malarici umani. *Policlinico*, sez. prat., 45 (1), 238.

—— (1939). Sullo sviluppo dei parassiti malarici. *Riv. Malariol.*, sez. II, 18, 149–54.

VEROLINI, F. (1949). Sulle modalità di comparsa del ciclo endoistiocitario del *P. gallinaceum* in polli previamente splenectomizzati. *Riv. Parassit.*, 10, 197–204.

VICH, A., & REY, F. (1939). Recientes adquisiciones en paludismo. Sobre la fase tisular de la monogonia. Interpretaciones erroneas de los primeros observadores. *Sem. méd. esp.*, 37, 626–8.

VILLALOBOS, E. (1940a). Sull' origine delle forme esoentrocitiche nel *P. gallinaceum*. *Riv. Parassit.*, 4, 113–16.

—— (1940b). Sull' origine delle forme esoeritrocitiche nel *P. gallinaceum*. *R. C. Ist. Sanit. pubbl.*, 4, 106–10.

VINCKE, I. H., & LIPS, M. (1950). Note sur la transmission cyclique du *P. berghei*. *Ann. Soc. belge Méd. trop.*, 30, 1605–11.

VINCKE, I. H., & PEETERS, E. (1953). Observations sur la transmission de sporozoïtes d'*Anopheles dureni* à des rongeurs sauvages et de laboratoire. *Ann. Soc. belge Méd. trop.*, 33, 87–93.

VOLTAIRE (1752). Micromegas, a philosophical tale. In: *Zadig and other tales* (Bohn's pop. ser.). London: G. Bell & Sons, 1928.

WALKER, A. J., & REID, J. A. (1953). Resistance to proguanil in the gametocytes and pre-erythrocytic forms of *P. falciparum*. *Trans. R. Soc. trop. Med. Hyg.*, 47, 580.

WARREN, A. J., & COGGESHALL, L. T. (1937). Infectivity of blood and organs in canaries after inoculation with sporozoites. *Amer. J. Hyg.*, 26, 1–10.

WASIELEWSKI, T. VON, & WÜLKER, G. (1918). Die Hämoproteus-Infektion des Turmfalken. *Beih. Arch. Schiffs- u. Tropenhyg.*, 22 (2), 113–212.

WENYON, C. M. (1926). *Protozoology*. Ballière, Tindall & Cox, London. 2 vols.

—— (1940). Abstract of CASINI, G. (1939). *Trop. Dis. Bull.*, 37, 58–9.

—— (1948). Discussion. *Trans. R. Soc. trop. Med. Hyg.*, 42, 33–4.

WHORTON, C. M., KIRSCHBAUM, W. R., PULLMAN, T. N., JONES, R., Jr., CRAIGE, B., Jr., ALVING, A. S., EICHELBERGER, L., & COULSTON, F. (1947). The Chesson strain of *P. vivax* malaria. I. Factors influencing the incubation period. *J. infect. Dis.*, 80, 223–7.

WINGSTRAND, K. G. (1947a). On some haematozoa of Swedish birds with remarks on the schizogony of *Leucocytozoon sakharoffi*. *K. svenska VetenskAkad. Handl.*, 3. ser. 24, No. 5, 1–31.

—— (1948). Further studies on *Leucocytozoon sakharoffi*. *K. svenska VetenskAkad. Handl.*, 3. ser., 24, No. 8, 1–17.

WISELOGLE, F. Y. (1946). (Ed.) *A survey of antimalarial drugs 1941–1945*. Ann Arbor, Mich.: J. W. Edwards, 2 vols.

WOLFSON, F. (1940a). Exo-erythrocytic schizogony associated with the wood-thrush strain of *P. cathemerium* in relation to the species of the host. *Amer. J. Hyg.*, 31 (sect. C), 26–35.

—— (1940b). Successful cultivation of avian plasmodia in duck embryos. *Amer. J. Hyg.*, 32 (sect. C), 60–1.

—— (1940c). Organisms described as avian *Toxoplasma*. *Amer. J. Hyg.*, 32 (sect. C), 88–99.

YOKAGAWA, S. (1942a). *Nippon Igaku*, 32, 589. Quoted by INOKI, S. (1951a).

—— (1942b). *Nettai Igaku*, 1, 7. Quoted by INOKI, S. (1951a).

—— (1942c). *Nettai Igaku*, 1, 13. Quoted by INOKI, S. (1951a).

YOELI, M. (1948). Non-pigmented malaria parasites in the bone marrow from a mixed infection of *Leishmania* and *P. vivax*. *Trans. R. Soc. trop. Med. Hyg.*, 42, 99–100.

YORKE, W. (1925). Further observations on malaria made during treatment of general paralysis. *Trans. R. Soc. trop. Med. Hyg.*, 19, 108–22.

—— (1931). Discussion. *Trans. R. Soc. trop. Med. Hyg.*, 24, 526–8.

YORKE, W., & MACFIE, J. W. S. (1924). Observations on malaria made during treatment of general paralysis. *Trans. R. Soc. trop. Med. Hyg.*, 18, 13–33.

YOUNG, M. D. (1953). Personal communication.

ZAIN, H. (1941a). Zum Ursprung der Endothelstadien des *P. gallinaceum*. *Arch. exp. Path. Pharmak.*, 197, 210–23.

—— (1941b). Verhalten der aus erythrocytären Parasitenformen hervorgegangen E-stadien des *P. gallinaceum*. *Arch. exp. Path. Pharmak.*, 198, 551–6.

—— (1941c). Zur Entstehung der Endothelformen der Vogelmalaria. (*P. gallinaceum*.) *Klin. Wschr.*, 20, 176–7.

ZAIN, H., & WOLF, A. (1943). Einfluss der Röntgenstrahlen auf die Entwicklung der Endothelstadien der Vogelmalaria (*P. gallinaceum*). *Dtsch. tropenmed. Z.*, 47, 68–71.
ZIEMANN, H. (1913). Ueber neuere Probleme der Tropenmedizin. *Z. Balneol.*, 6, 1–10.
ZUCKERMAN, A. (1946). Infections with *P. gallinaceum* in chick embryos induced by exo-erythrocytic and blood stages. *J. infect. Dis.*, 79, 1–11.

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